



Life Sciences

Program Tasks and Bibliography

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I. Introduction

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TASK BOOK INTRODUCTION FOR FY 1997

The NASA Life Sciences Division serves the Nation's life sciences community by managing all aspects of U.S. space-related life sciences research and technology development. The activities of the Division are integral components of the Nation's overall biological sciences and biomedical research efforts. However, NASA's life sciences activities are unique, in that space flight affords the opportunity to study and characterize basic biological mechanisms in ways not possible on Earth. By utilizing access to space as a research tool, NASA advances fundamental knowledge of the way in which weightlessness, radiation, and other aspects of the space flight environment interact with biological processes. This knowledge is applied to procedures and technologies that enable humans to live and work in and explore space and contributes to the health and well-being of people on Earth.

The Office of Life and Microgravity Sciences and Applications (OLMSA) is responsible for planning and executing research stimulated by the Agency's broad scientific goals. OLMSA's Life Sciences Division is responsible for guiding and focusing a comprehensive program of flight and ground-based tasks. This document, the Life Sciences Program Tasks and Bibliography for FY 1997 (October 1996-September 1997), includes all peer reviewed projects funded by OLMSA's Life Sciences Division during FY 1997. This document is published annually and made available to scientists in the space life sciences field both as a printed document and as an interactive internet web site (<http://peer1.idi.usra.edu>). The information provided in the Task Book is used in reports to the NASA Associate Administrator, the Office of Management and Budget, and to the United States Congress.

In this book, flight tasks are organized by flight mission/program while ground-based tasks are divided into fourteen elements within five major programs. The on-line Task Book offers the option of searching all tasks by principal investigator's name, project title, keyword, Life Sciences Division classifications (i.e., program, element), scientific discipline, and flight information (where applicable). A complete listing of scientific programs and elements, as well as flight missions/programs, is provided on page I-5.

Please note the following:

- The FY 1997 Task Book includes graduate student research projects (GSRPs).
- The FY 1997 Task Book includes flight projects in Definition Phase. These projects received money in FY 1997 to further develop plans for flight-based research which may greatly contribute to the long-term scientific goals of the Life Sciences Division. *The proposed investigators were not under contract with NASA in FY 1997 to perform scientific experimentation in conjunction with these projects.*
- *Funding amounts in the FY 1997 Task Book represent approximate dollars obligated from the FY 1997 Life Sciences Division budget rather than actual dollars spent.* In general, the amounts listed for ground-based research represent approximations/projections as of July 1, 1997, and the amounts listed for flight research represent approximations/projections as of June 1, 1997. Funding information for any individual task was provided by the appropriate monitoring center (HQ, ARC, JSC, or KSC).

The Life Sciences Division wishes to thank Information Dynamics, Inc. and Universities Space Research Association personnel and in particular, recognize John Nelson (task book review process and publication manager), Jennie Moehlmann, Bill Wilcox, and Lori Tyahla for their efforts in the development, compilation, and publishing of this report. Gratitude is also expressed to the following people who were responsible for coordinating flight task data delivery from NASA field centers: Bonnie Dalton (point of contact), Amy Chu, and Alison French at ARC; Helen Lane (point of contact), Elisa Allen, Sharon Jackson, and Bonnie Meadows at JSC; and Cindy Martin (point of contact), Elise Blaese, Doug Gruendel, and Dave Reed at KSC.

FY 1997 PROGRAM RESEARCH TASK SUMMARY:**Overview Information and Statistics**

Total Number of Principal Investigators (Excluding GSRP Investigators):	350
Total Number of GSRP Investigators:	24
Total Number of Co-Investigators:	532
—	
Total Number of Principal Tasks (Excluding GSRPs):	436
Total Number of GSRPs:	24
—	
Total Number of Bibliographic Listings:	1,749
• Proceedings Papers:	179
• Journal Articles:	697
• Journal Abstracts:	216
• <u>NASA Tech Brief</u> Articles:	24
• Science/Technical Presentations:	482
• Books/Chapters:	128
• Theses/Dissertations:.....	23
Total Number of Patents Applied for or Awarded:	10
—	
Number of Students Funded:	866
Number of Post-Doctoral Associates Funded:	306
—	
Number of States with Funded Research (including District of Columbia):	41
—	
FY 1997 Life Sciences Division Budget:	\$97.4 Million

Number of Tasks listed by Program and Element

<i>Program</i>	<i>Element</i>	<i>Ground</i>	<i>Flight</i>	<i>Total</i>
AHST	Advanced Environmental Monitoring and Control	16	2	18
	Advanced Life Support	16	2	18
	Space Human Factors Engineering	10	0	10
	Total	42	4	46
BR&C	Behavior and Performance	6	8	14
	Environmental Health	7	9	16
	Radiation Health	29	3	32
	Space Physiology and Countermeasures	93	54	147
	Total	135	74	209
GB&E	Cellular and Molecular Biology	20	14	34
	Developmental Biology	8	30	38
	Education	1	0	1
	Plant Biology	18	21	39
	Remote Sensing and Ecology	1	0	1
	Total	48	65	113
GSRP		24	0	24
NSCORT		68	0	68
Total Tasks		317	143	460

Number of Flight Tasks listed by Flight Mission/Program:

<i>Flight Mission/Program</i>	<i>Number of Tasks</i>
Bion	6
Biorack	9
Definition	10
LMS	12
Mir	37
Neurolab	24
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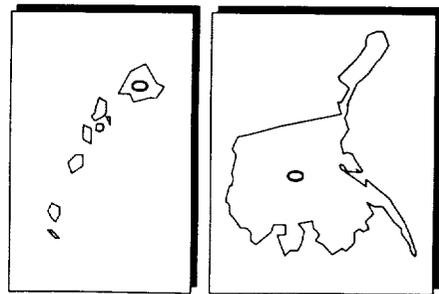
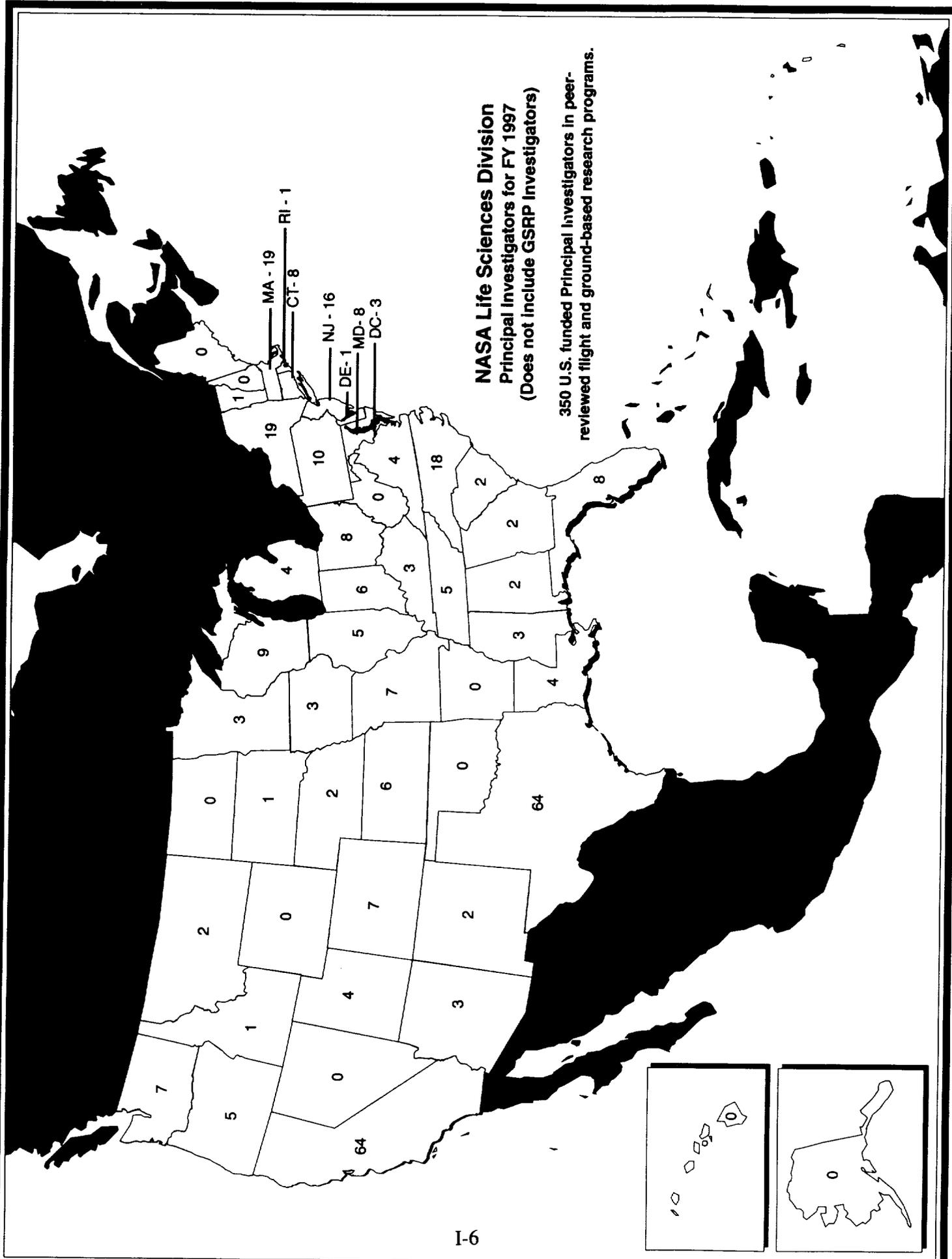
Please note the following:

Investigators are counted only once even if they work on multiple tasks.

“Total number of Co-Investigators” excludes duplicates and individuals already counted as Principal Investigators.

Metrics include tasks and investigators under no-cost extensions as well as individual tasks and investigators which appear collectively within a single Task Book entry (e.g., NSCORTs).

Individual bibliographic entries are counted only once even if they appear in conjunction with multiple tasks. Submissions not yet accepted for publication during FY97 are not included in bibliographic metrics.



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Functional Neuromuscular Adaptation to Spaceflight

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Funding:

UPN/Project Identification: 106-30-46
Initial Funding Date: 1991
Students Funded Under Research: 9
FY 1997 Funding: \$400,179

Solicitation: 88-OSSA-8
Expiration: 1998
Post-Doctoral Associates: 3

Flight Information:

Experiment ID: 8809A13
Flight Assignment: Bion 11 (December 1996)
Responsible NASA Center: Ames Research Center

Task Description:

Monkeys were trained to perform a specific psychomotor task using a loaded foot lever and trained to walk on a motor driven treadmill. Electromyographic (EMG) signals from leg flexor and extensor muscles, force developed by the medial gastrocnemius muscle and foot lever information, were recorded during these behaviors before and after flight. Additionally, EMG and force recordings were made over 24 hour periods of normal cage activity before and after flight. Inflight recordings were made only during the psychomotor task.

These data will be used to evaluate the effects of microgravity on normal motor function, the changes that occur with adaptation to a microgravity environment, both in the microgravity environment and upon return to Earth and the readaptation that occurs on return to Earth.

Pre-, post-, and inflight recordings were completed during FY97. These video recordings of treadmill locomotion shortly after return to Earth show considerable postural instability, even when the monkeys were in a sitting position. Periods of muscle tremor were also evident. Analysis of the locomotion EMG data is nearing completion. Results suggest a decrement in soleus EMG amplitude following space flight with a lesser decrement in the restraint control animals. A decrement in the medial gastrocnemius and VL EMG amplitudes was observed postflight whereas after restraint, EMG amplitudes increased transiently. The EMGs also exhibited periods of tremor-like activity. MG tendon force increased in postflight locomotion tests.

The first stages of analysis of the 24 hour EMG data are complete. Hourly integrals show a circadian cycle in most animals. Additional processing is required for some records where noise contaminated some periods of recording. Joint probability density analysis of soleus and gastrocnemius EMGs before, during and after flight

from one animal performing the psychomotor task has been completed. The results from this animal show a slight shift in EMG activity from soleus to medial gastrocnemius but not so pronounced as that observed after the Bion 10 flight. During flight there was a marked uncoupling of activity in these two muscles which are normally coactive.

Our work to date provides a significant body of data indicating disruption of the normal control of muscle activation. JPDs during the psychomotor task and reciprocal changes in amplitude of soleus and medial gastrocnemius activity during locomotion indicate alterations in the recruitment patterns of muscle and motor units after space flight.

This project addresses problems related to neuromuscular diseases as well as the problem of muscle atrophy as occurs in response to space flight. Further, these studies contribute to our understanding of the control of movement in the unique space flight environment and have considerable bearing on the control of movement, such as standing and maintaining upright posture in the aging population. The proposed research should give us a considerably clearer understanding of the physiological signals which may contribute to the maintenance of muscle mass. For example, the activity levels in muscles of the legs will be monitored during normal activities at normal gravitational loading as well as in the microgravity environment. These data should indicate the importance of activity in maintaining normal mass and functional properties of flexor and extensor muscles. The role of activity of specific muscles in maintaining normal levels of control of movement also will be determined. One of the major advantages of the proposed experiments in efforts to understand basic biological processes is that the normal neuromuscular system will be studied in an abnormal physiological environment, i.e., the altered function is caused by an altered environment, not an altered capability of the physiological system being studied as would be the case with surgical or pharmacological manipulation.

Each phase of these experiments has important implications on the optimization of rehabilitative care in addressing problems related to neuromuscular dysfunction as well as some aspects of hormonal function. These results could have a fundamental and large impact on currently accepted approaches to the rehabilitation of a number of medical conditions in which a person remains in bed for prolonged periods, in individuals with compromised neuromuscular systems, and in the aging population.

Observations in the Bion and LMS programs have emphasized the importance of gravitational loading in providing appropriate sensory signals for correct operation of the motor control systems of the spinal cord. These findings have prompted us to investigate therapeutic interventions emphasizing weight support in spinal cord injured and stroke patients in our NIH sponsored rehabilitation research. Initial results from this work show remarkable promise, even to the extent of inducing stepping in spinal cord injured patients diagnosed as completely paralyzed.

FY97 Publications, Presentations, and Other Accomplishments:

Edgerton, V.R., Roy, R.R., Harkema, S.J., Hodgson, J.A., and Dobkin, B.H. "How studies of adaptations to spaceflight contribute to understanding the plasticity of spinal cord injury." Proceedings of the 48th IAA Congress (In Press).

Recktenwald, M.R., Hodgson, J.A., Roy, R.R., Garfinkel, A., and Edgerton, V.R. "Effects of spaceflight on quadrupedal locomotion of rhesus monkeys after return to 1g." (Abstract) Soc. Neurosci. Abstr., 23: 760 (1997).

Roy, R.R., Hodgson, J.A., Aragon, J., Day, M.K., Kozlovskaya, I., and Edgerton, V.R. "Recruitment of the rhesus soleus and medial gastrocnemius before, during and after spaceflight." J. Grav. Physiol., 3(1), 11-16 (1996).

Effect of Weightlessness on Single Muscle Fiber Function in Rhesus Monkeys

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-30-46
 Initial Funding Date: 1990
 Students Funded Under Research: 3
 FY 1997 Funding: \$91,000

Solicitation: 88-OSSA-8
 Expiration: 1998
 Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 8913020
 Flight Assignment: Bion 11 (December 1996)
 Responsible NASA Center: Ames Research Center

Task Description:

Our long-term objectives are to understand the cellular mechanisms of muscle contraction and to determine how zero gravity affects muscle function and the physical work capacity. Although it is well known that zero-G induces considerable limb muscle atrophy, little is known about how weightlessness alters cell function. In this proposal, we will utilize the single skinned fiber and single freeze-dried fiber preparations to evaluate how weightlessness alters the functional properties of single fast and slow striated muscle fibers. Muscle biopsies will be obtained from the soleus and gastrocnemius muscles of the Rhesus monkey before and as soon as possible after the zero-G flight (Bion). The biopsies will be divided, and one-half will be quick frozen in liquid nitrogen and the other placed in skinning solution (-20° C). The frozen samples will be freeze-dried and stored under vacuum (-80° C) for subsequent biochemical analysis, while the skinned fiber bundle will be used to study the physiological properties of individual fast- and slow-twitch fibers.

Physiological studies will test the hypothesis that zero-G causes fiber atrophy, a decreased peak force (Newtons), tension (Newtons/cross-sectional area) and power, an elevated peak rate of tension development (dp/dt), and an increased maximal shortening velocity (V_0) in the slow type I fiber, while changes in the fast-twitch fiber will be restricted to atrophy and a reduced peak force. For each fiber, we will determine the peak force (P_0), V_0 , dp/dt, the force-velocity relationship, peak power, the power-force relationship, the force-pCa relationship, and fiber stiffness.

Biochemical studies will assess the effects of weightlessness on the enzyme and substrate profile of the fast- and slow-twitch fibers. We predict that zero-G will increase resting muscle glycogen and glycolytic metabolism in the slow fiber type, while the fast-twitch fiber enzyme profile will be unaltered. The increased muscle glycogen will in part result from an elevated hexokinase and glycogen synthase. The enzymes selected for study represent markers for mitochondrial function (citrate synthase and b-hydroxyacyl-CoA dehydrogenase), glycolysis

(Phosphofructokinase and lactate dehydrogenase), and fatty acid transport (Carnitine acetyl transferase). The substrates analyzed will include glycogen, lactate, adenosine triphosphate, and phosphocreatine.

Following each of the physiological and biochemical studies described above, a section of the fiber will be loaded on a 5% SDS-PAGE gel to assess the myosin heavy chain isozyme profile. This analysis will allow us to group the studied fibers as slow- or fast-twitch, and determine if space flight had any effect on the type of myosin expressed in a given fiber type. In order to evaluate the myosin light chain and regulatory proteins, we will also conduct 12% SDS-PAGE analysis on single fibers isolated from each biopsy sample.

In FY97, the 14 day BION 11 flight took place and we acquired muscle biopsy samples pre- and post-flight from the soleus and gastrocnemius muscles of the 2 flight monkeys as well as growth and environmental control groups. The samples were divided into 3 sections which allowed studies to be conducted on single fiber physiology, biochemistry, and structure. The results were compared to those obtained in humans following a 17 day flight (LMS). The slow type I fibers of the soleus and gastrocnemius muscles underwent a 10% and 22% decline in fiber size, respectively. Atrophy of the fast fiber types was only observed in the soleus. However, all fiber types from both muscles showed a significant loss in peak force with the largest decrease (48%) observed in the type I fiber of the gastrocnemius. The decline in force was not entirely explained by the fiber atrophy as the force per cross-sectional area also showed a significant loss in all fiber types. The growth controls showed no significant changes in cell physiology, and thus growth did not influence the space flight results. The ground chair controls did show fiber atrophy, however, unlike space flight the fibers did not undergo a decline in peak force per cross-sectional area. Thus the changes with space flight were unique and not simply a result of a reduced physical activity. Although the zero-G induced cellular changes in limb skeletal muscle were for the most part similar between man and monkey, there were 2 notable exceptions. In humans, zero-G induced a significant increase in the maximal shortening speed of both the slow- and fast-twitch fibers. As a result of this adaptation, the fall in peak power (the product of force and velocity) was significantly less in man compared to monkeys. A second difference between species was the manner in which the slow type I fibers of the gastrocnemius responded to weightlessness. In man, this fiber type showed no atrophy or loss in force, and as a result of the increase in velocity, peak power showed a small increase. In contrast, the slow fiber of the monkey gastrocnemius showed significant atrophy, and lost force and power. Our hypothesis is that these differences can be explained by the countermeasures employed by the LMS crew, and by postural differences. Future studies will be needed to establish the relative importance of the zero-G removal of muscle loading versus muscle length changes in inducing the functional decline in limb muscle function, and whether or not a new steady state is ultimately reached at some level of reduced performance. Additionally, high intensity (isotonic and isometric) countermeasures must be tested for their effectiveness as the aerobic procedures currently used are not preventing fiber atrophy or the loss of force and power.

A major goal of this research is to elucidate the functional changes associated with zero-G induced muscle wasting and to use this information in the development of effective exercise countermeasures. The program is essential to our ability to explore the universe and work successfully in space. Stated another way, we simply cannot embark on long-term space travel until we can understand and prevent muscle wasting. Similar types of muscle atrophy occur on Earth in various muscle diseases and during the normal aging process. This work will provide an increased understanding of basic muscle function, and how it is deleteriously altered with inactivity. Furthermore, it will provide the basic knowledge needed for the development of new exercise protocols and strategies that should be more effective than current procedures in slowing atrophy associated with the aging process. Since one of the main problems encountered by older adults is weakness which leads to debilitating falls, these modalities will improve the quality of life and lead to considerable savings in medical costs.

FY97 Publications, Presentations, and Other Accomplishments:

Widrick, J.J., Romantowski, J.G., Karhanek, M., and Fitts, R.H. "Contractile properties of rat, rhesus monkey, and human type I muscle fibers." *Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 41)*, 272, R34-R42 (1997).

Homeostatic and Circadian Responses of Rhesus Monkeys During Space Flight

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Funding:

UPN/Project Identification: 106-30-46
Initial Funding Date: 1993
Students Funded Under Research: 2
FY 1997 Funding: \$126,000

Solicitation: 88-OSSA-8
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Flight Assignment: Bion 11 (December 1996)
Responsible NASA Center: Ames Research Center

Task Description:

Mammals have developed the ability to adapt to most variations encountered in their everyday environment. However, throughout the evolution of life on Earth, living organisms have been exposed to the influence of both the unvarying level of Earth's gravity and the natural 24-hour day resulting from the rotation of the planet. As a result, changes in either or both of these factors produce adaptive responses which are not completely understood. In particular, homeostatic systems such as sleep, temperature regulation, and biological rhythms are influenced. The adaptations that occur in these systems appear to produce deleterious results in individuals exposed to long-term temporal isolation or altered gravitational environments. This program will examine the influence of microgravity on these systems in rhesus monkeys. Further, the homeostatic regulation of these variables as influenced by light and dark will be studied during space flight. The results should provide data on the adaptation of these systems to this environment, as well as information for supporting crew operations in microgravity.

The Bion 11 flight scheduled for mid-summer 1996 was slipped to late December. Pre-flight preparations were also moved to accommodate that schedule. In order to support the development of this flight activity, we had participated in meetings and discussions concerning flight and ground-based experimentation and assisted in the generation of supporting documents and development of sensors. Among these activities were the writing and editing of the Experiment Management Plans (EMP) for the three Regulatory Disciplines as well as the Integrated EMP. We also developed and presented the Regulatory Team science objectives to the Bion Program Review Panel.

Prior to the launch of Bion 11, each flight candidate was the subject of a 3-day capsule test. During these tests, all data were recorded as they would be during flight. Thus, these tests provided a short, preflight baseline for the Circadian Rhythm and Thermoregulation experiments. A member of our team traveled to Moscow and actively participated in these tests. We have begun our analysis of these data.

In addition to the capsule test, each monkey from the flight pool also participated in a 4-day metabolic test. Doubly labelled water was given at the start of each test and serial urine collections were made to allow for the determination of total body water and energy expenditure. Analysis of these biosamples is proceeding. Again, a member of our team traveled to Moscow to take part in these tests.

The Bion 11 flight was launched on December 24, 1996 and recovered on January 7, 1997. Pre-launch preparations took place in Moscow and Plesetsk. A member of our team traveled to Moscow to help support these activities.

Two postflight control experiments were conducted, one at R+17 and one at R+45. The flight animals were to be the subjects of the R+45 experiment. We actively participated in both of these control experiments in Moscow.

We have received all of the electronic data collected during the flight, R+17 and R+45 experiments, as well as all the urine samples. Analysis of these data is on going. We have noted that, as had been seen in Bion 10, during space flight, the timing of the brain temperature rhythm is delayed compared to the control.

The study of physiology and behavior is frequently divided into the examination of specific control systems. Similarly, in the control of such systems, it is also vital to recognize that these systems are integrated and function together interdependently. Thus, to fully understand a function such as temperature regulation, one must view control of temperature regulation at various levels. For example, temperature regulation is known to interact with a variety of other systems, including sleep, respiration, endocrine, and cardiovascular. Moreover, there is a prominent temporal component; i.e., a circadian temperature rhythm. Physiological regulation as well as behavioral performance capacity can be severely impaired when temporal information within the organism is not sufficient to maintain internal synchrony between and/or within physiological control systems. During desynchronization, psychotic states may be induced and performance capabilities of simple tasks may diminish in rhesus and humans. These pathologies may arise not only in environments without time cues, such as constant light (or constant dim light found in many of today's intensive care units), but also with shifts in time zones, shift work, and in aging individuals where internal temporal coupling appears weakened. Narcolepsy is a class of diseases in which daytime sleep attacks or Rapid Eye Movement (REM) sleep onset can occur. Some of these individuals display a loss of circadian patterns of REM sleep distribution. Further, when the individuals are tested for sleep latencies throughout the 24-hour day, there is often lack of circadian variation in the sleep latency as compared with the normal subjects. Other instances have been studied in which individuals cannot synchronize themselves with their environment and maintain a 24-hour day, but rather free-run with a circadian 25-hour day. Phase relationships between sleep and body temperature cycles may play a key role in the oscillations between mania and depression in manic-depressives. An additional syndrome with links to altered circadian function is winter depression. The remission of the depression is simultaneous with the correction of the phase irregularity. Several lines of evidence demonstrate the sensitivity of the sleep control mechanism to the dynamic environment. The early Gemini flights showed changes in sleep duration and spectral power density of the electroencephalogram (EEG) early in the flight. On the Apollo and Skylab missions, sleep was also modified during initial exposure to space flight. Sleep onset has been a problem both for some Soviet cosmonauts and American astronauts, sometimes requiring the use of sleeping pills. Early reports on sleep stages assumed that slow wave sleep content is increased and REM sleep decreased. However, the recent Spacelab 1 findings of increased REM activity contradict this. There is a possibility that pre-flight sleep deprivation of Spacelab 1 subjects may have artificially increased REM sleep by well-documented rebound phenomenon. On a recent Mir mission, an individual showed a phase delay in this temperature rhythm and a diminished performance capacity that was linked to a decrease in fine motor control.

In summary, these investigations will provide basic information on function of homeostatic control systems in primates. This information will form the basis for the design of countermeasures used to prevent the performance, psychological, and health decrements that occur when these systems are adversely affected. These countermeasures will not only be of the utmost importance as humans extend the length of time of exposure to space flight, but should also prove useful to people on Earth who suffer from homeostatic, particularly circadian, imbalances.

FY97 Publications, Presentations, and Other Accomplishments:

Fuller, C.A., Hoban-Higgins, T.M., Klimovitsky, V.Y., Griffin, D.W., and Alpatov, A.M. "Primate circadian rhythms during spaceflight: Results from Cosmos 2044 and 2229." J. Appl. Physiol., 81, 188-193 (1996).

Stein, T.P., Dotsenko, M.A., Korolkov, V.I., Griffin, D.W., and Fuller, C.A. "Energy expenditure in rhesus monkeys (*Macaca mullata*) during spaceflight using doubly labelled water ($2\text{H}_2^{18}\text{O}$)." J. Appl. Physiol., 81, 201-207 (1996).

Bone and Lean Body Mass Changes Following Space Flight

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Funding:

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Solicitation: 88-OSSA-8

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Expiration: not available

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Flight Information:

Experiment ID: 284029

Flight Assignment: Bion 11 (December 1996)

Responsible NASA Center: Ames Research Center

Task Description:

Post flight lean mass and bone density will be compared to pre-flight values for the flight primates and the control primates. Data was collected on the Hologic QDR 1000 DEXA scanner located at IBMP in Moscow. Comparison will be made between the flight and the control primates and between muscle and bone for changes incurred during the flight period. Data will be shared with scientists performing histologic measurements to determine relationship of densitometric changes to changes at the tissue and cellular levels.

Data collection was completed on pre-flight and post-flight DEXA scans.

Previous flights involving animals and humans aboard Russian (Mir, Cosmos) and American spacecraft (Skylab, Spacelab) have documented that significant bone and muscle atrophy occurs during weightlessness requiring the development of effective and efficient countermeasures. The losses during space flight are believed to result from the reduced forces on the musculoskeletal system, analogous to the changes from inactivity in 1-G. The loss of bone mineral with aging occurs in both men and women, resulting in a significant public health problem in the United States and other countries of the world. It is estimated that the medical cost of osteoporosis in the United States is 7 to 10 billion dollars per year. Although the exact causes of osteoporosis are unknown, one important risk factor is disuse. Men and women become less active as they grow older, and that may play an important role in the osteopenia in the elderly and in patients immobilized for medical reasons. Similarly muscle atrophy is an important component of many disease states as well as aging and, therefore, understanding the role of disuse versus other causes is important for elucidating the physiological mechanism of muscle

atrophy. Comprehending these mechanisms is important for developing effective countermeasures to preserve bone and muscle function in disease conditions as well as space flight.

Behavior and Performance Project

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1987
Students Funded Under Research: 5
FY 1997 Funding: \$231,304

Solicitation: 88-OSSA-8
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 8808A09
Flight Assignment: Bion 11 (December 1996)
Responsible NASA Center: Ames Research Center

Task Description:

Behavior is an overt manifestation of underlying physiology, and to the degree that biological systems are compromised by space flight, it is reasonable to expect at least subtle behavioral alterations. Exacerbated physiological compromise may well result in serious psychological consequences, evidenced either as changes in the psychological well-being of the individual or as manifest disruptions in performance. The Behavior and Performance Project was designed to address these important aspects of mission success, and has four primary goals: 1) to support and assess the psychological well-being of the research animals; 2) to examine the effects of space flight on cognitive and motor performance; 3) to relate behavioral measures to physiological data from other disciplines; and 4) to provide expertise and support for training the monkeys to perform the tasks for all flight experiments.

Behavior and Performance Project scientists have developed an apparatus, the Psychomotor Test System (PTS), in which monkeys respond to computer-graphic stimuli by manipulating a joystick in accordance with task demands. The PTS has been demonstrated to be highly effective for improving and assessing psychological fitness. Supporting and monitoring the psychological well-being of nonhuman primates maintained for research purposes is mandated by scientific, ethical, and legal considerations. Using this device and a variety of behavioral measures, we will provide environmental enrichment for, and assess the psychological well-being of, rhesus monkeys before, during, and after space flight research.

We also propose to use the PTS to identify alterations in cognitive and psychomotor performance that result from space flight. A battery of assessment tasks will be administered before and after the flight, and measures of memory, attention, perception, learning, and psychomotor functioning will be analyzed for evidence of changes that result from microgravity or other space flight-relevant variables.

These psychological data will then be related to physiological measures obtained by scientists representing other disciplines. We anticipate that this bio-behavioral integration (e.g., of performance data with measures from muscle or regulatory physiology) may reveal overt behavioral indices that are diagnostic of underlying physiological compromise.

Finally, we have assumed an active role in training the rhesus monkeys for various aspects of the space flight research. We developed and implemented a curriculum of tasks that instate PTS skills. We have also provided expertise for improving the training of monkeys to execute behaviors necessary for other disciplines (e.g., treadmill locomotion, foot-pedal responding).

Using software and procedures developed at the Sonny Carter Life Sciences Laboratory at Georgia State University, a colony of monkeys was trained in Moscow in preparation for the Bion 11 space flight. This training protocol was effective in establishing two important types of behavior for the flight. First, the monkeys learned to manipulate a joystick so as to respond to computer-generated stimuli in accordance to the demands of tasks in the PTS. Second, we worked with colleagues from the muscle physiology discipline to establish patterns of treadmill locomotion by rhesus monkeys that could be studied before and after the space flight. In December, 1996 we participated in the Bion 11 space flight, which was recovered in January, 1997. We obtained preflight and postflight PTS data, although little PTS data were obtained shortly after flight. We also coded psychological well-being from behavioral indices for all monkeys before and after the flight. Pre- and postflight locomotion data were also recorded. Further, PTS, well-being, and locomotion data were collected in two control studies. The analysis of these important data continues today.

In addition to these activities directly related to space flight, we have continued to study the basic mechanisms of cognition and well-being, using monkeys at the Sonny Carter Life Sciences Laboratory at Georgia State University. Ongoing studies in learning, memory, metacognition, attention, and psychological well-being provide the background and parametric manipulations necessary for integrating our flight results into the psychological literature.

Finally, we have continued to support the spin-off applications of the technologies and procedures developed for space life sciences research. The quest to apply PTS tasks and procedures to the education and testing of children with mental retardation, autism, and attention deficit and hyperactivity disorders has been expanded. Similarly, we have explored options for increasing the utility of the PTS as a general-purpose laboratory test and enrichment system. Finally, this fiscal year has seen the successful application of the PTS and the training protocols developed for the Rhesus and Bion Projects to even more nonhuman animal species.

This research is motivated by two pressing needs in space life sciences: (1) the need to understand and address the physical and psychological consequences of space flight, subsumed under the title "space adaptation syndrome;" and (2) the legal, ethical, and scientific mandate to provide for and to assess the psychological well-being of nonhuman primates before, during, and after each flight in which they serve as research subjects. Moreover, the research promises to produce several definite Earth benefits. First, the relation between behavior and corresponding biological systems will be illuminated through space flight research. Indeed, the basic science benefits of space flight research reported by any other discipline can be said also to improve our understanding of the relation between behavioral and biological systems.

We have already witnessed numerous Earth benefits from the development of the PTS. For example, the system has proven to be a remarkably effective tool for comparative psychological research. Many primate species have been trained and tested with the system, and their data have in many instances revolutionized the understanding of the continuities in psychological processes among monkeys, apes, and humans. Additionally, the test device has proven to be very useful as a general laboratory enrichment device. At a time when laboratories everywhere are working to satisfy the federal requirements governing the psychological well-being of captive primates, the PTS has become an acclaimed and popular option. For these reasons, over three dozen laboratories world-wide have requested and received assistance in constructing and using PTS for their research and enrichment needs.

The PTS has also been used in educational applications—with college students as well as school-aged children. For example, many domains of development and skill frequently have not been accessible for some youths with mental retardation and impaired oral language abilities. The PTS affords a battery of computer-facilitated nonverbal tasks that employ methodology that is appropriate for the communicative abilities of these children and young adults. We have utilized the PTS to examine performance in perceptual-motor, cognitive-learning, and neuropsychological function. For example, a recent study of the visual short-term memory skills of students with moderate mental retardation revealed that even lengthy retention intervals were tolerated with little difficulty. Data such as these underscore the advantage of studying heretofore untapped skills of persons with cognitive and linguistic disabilities.

FY97 Publications, Presentations, and Other Accomplishments:

Filion, C.M., Washburn, D.A., and Gulledge, J. P. "Can monkeys respond to invisible displacements?" *J. Comp. Psych.*, 110, 386-395 (1996).

Gulledge, J.P. and Washburn, D.A. "Visual and spatial memory performance by humans, apes, and monkeys." (Poster) Meeting of the Southeastern Psychological Association, Atlanta, GA (April, 1997).

Gulledge, J.P. and Washburn, D.A. "Why monkeys can't CONCENTRATE: An investigation of visual-spatial memory." Meeting of the Southern Society for Philosophy and Psychology, Atlanta, GA (March, 1997).

Hopkins, W.D., Washburn, D.A., and Hyatt, C.W. "Video-task acquisition by rhesus monkeys (*Macaca mulatta*) and chimpanzees (*Pan troglodytes*): A comparative analysis." *Primates*, 37, 197-206 (1996).

Putney, R.T. and Washburn, D.A. "The factors of attention: A "meta-analysis."" Meeting of the Southeastern Psychological Association, Atlanta, GA (April, 1987).

Rumbaugh, D.M. and Washburn, D.A. "Discrimination learning set and transfer" in "Encyclopedia of Comparative Psychology." Edited by: Greenberg, G. and Haraway, M. New York: Garland Publishing (In Press).

Rumbaugh, D.M., Savage-Rumbaugh, E.S., and Washburn, D.A. "Toward a new outlook on primate learning and behavior: Complex learning and emergent processes in comparative perspective." *Japanese Psychological Research, Special Issue: Cognition and behavior of nonhuman primates*; Jitsumori, M., Matsuzawa, T., and Kojima S. (Guest Eds.), 38, 113-125 (1996).

Rumbaugh, D.M., Washburn, D.A., and Hillix, W.A. "Respondents, operants, and emergents: Toward an integrated perspective on behavior" in "Learning as a Self-Organizing Process." Edited by: Pribram, K. and King, J. Hillsdale, NJ: Erlbaum, pp 57-73 (1996).

Shields, W.E., Smith, J.D., and Washburn, D.A. "Uncertain responses by humans and rhesus monkeys in a psychophysical same-different task." *J. Exp. Psych.*, (In Press).

Shields, W.E., Smith, J.D., and Washburn, D.A. "Memory monitoring by rhesus monkeys." (Poster) Annual meeting of the Psychonomic Society, Chicago, IL (November, 1996).

Smith, J.D., Shields, W.E., Allendoerfer, K.A., and Washburn, D.A. "Metamemory-like processes by monkeys and humans." *J. Exp. Psych.*, (In Press).

Smith, J.D., Shields, W.E., Schull, J., and Washburn, D.A. "The uncertain response in humans and animals." *Cognition*, 62, 75-97 (1997).

Washburn, D.A. "Animal cognition: An introduction to modern comparative psychology." *Trends in Cognitive Sciences*, 1, 154 (1997).

Washburn, D.A. "What monkeys can do." *Teaching of Psych.*, 24, 61-35 (1997).

Washburn, D.A. "Recognition and recall components of monkey memory." (Poster) Meeting of the American Psychological Society, Washington, DC (May, 1997).

Washburn, D.A. and Astur, R.S. "Comparative investigations of visuospatial memory: Rehearsal in the sketchpad?" *Memory and Cognition* (In Press).

Washburn, D.A. and Rumbaugh, D.M. "If faster is smarter, why are we slower: A comparative perspective on intelligence and processing speed." *Am. Psychologist* (In Press).

Washburn, D.A. and Rumbaugh, D.M. "Processing speed and intelligence: Is faster better?." Meeting of the Psychonomic Society, Chicago, IL (November, 1996).

Washburn, D.A., Gullledge, J.P., and Rumbaugh, D.M. "The heuristic and motivational value of video reinforcement." *Learning and Motivation* (In Press).

Washburn, D.A., Rumbaugh, D.M., and Richardson, W.K. "Apparatus" in "Encyclopedia of Comparative Psychology." Edited by: Greenberg, G. and Haraway, M. New York: Garland Publishing (In Press).

Washburn, D.A., Sevcik, R.A., Rumbaugh, D.M., and Ronski, M.A. "Educational applications of the psychomotor test system." *Proceedings of the AIAA Life Sciences and Space Medicine Conference and Exhibit* (pg. 72-73) American Institute of Aeronautics and Astronautics.

An Evaluation of Collagen Metabolism in Non-Human Primates associated with the BION Space Program-Markers of Urinary Collagen Turnover and Muscle Tissue Collagen Types

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Funding:

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Solicitation: AO-84

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 119,000

Flight Information:

Flight Assignment: Bion 11 (December 1996)

Responsible NASA Center: Ames Research Center

Task Description:

Metabolic by-products of tissue metabolism, measured in urine of astronauts and cosmonauts, suggested an increased degradation of connective tissues during short duration exposure to microgravity. In addition, an increased concentration of hydroxyproline (a connective tissue marker for intracellular and extracellular collagen degradation) in urine was documented in flight studies.

Collagen is the most abundant protein in the body and is the principle structural protein of connective tissues. Connective tissues are important in maintaining the stability of joints and the body's structural integrity (muscle, bones, cartilage, tendons, and ligaments) and also are involved in the translation of mechanical stresses to bones. Therefore, alterations in the load environment (ground reaction forces and muscle forces) by space flight will modify connective tissue metabolism. The increased urinary excretion of hydroxyproline and mineral salts in astronauts is evidence that degradation of connective tissue seems to be enhanced by weightlessness. Sources of connective tissue loss include muscle, bone, cartilage, tendons, and ligaments. Unfortunately, an assessment of collagen loss (index of connective tissue degradation) has been limited because of the technological limitations (MRI, Bone Scans, intracellular metabolites). Only recently, our laboratory and a few others (Great Britain) have measured the presence of a total collagen cross-links in plasma, serum, and urine. Therefore, a considerable effort needs to be directed toward the assessment of collagen loss by using a non-invasive marker of mature collagen degradation (hydroxylslypyridinoline and lysylpyridinoline).

The idea of utilizing non-invasive markers for evaluating the connective tissue response to microgravity has merit. Non-invasive markers have been clinically tested in cases that involve first level analysis for changes in connective tissue metabolism. Furthermore, the rationale for prescribing marker usage was based upon the fact that changes in connective tissue metabolites precede alterations in tissue macrostructure. Therefore, the purpose of this section of the investigation is to use a battery of non-invasive metabolic measurements which evaluates the connective degradation pre- and post-flight.

PostFlight BION 11 Sample Processing FY96-FY97:

Non-invasive bio-markers of collagen metabolism were measured to evaluate the connective tissue response of non-human primates in the BION 11 Space Program. Daily (24 hr.) urine collections were taken from eleven monkeys during pre-flight preparation and during the post-flight recovery period following fourteen days of space flight.

Urine specimens from the BION 11 project were shipped on dry ice, counted, inventoried, and immediately stored at -85° C until further analysis. Preflight samples consisted of samples from June through October, 1996. Postflight samples consisted of samples from January 7th (immediately postflight) to March 1997. The samples were assigned a random number and analyzed blindly to avoid any measurement bias.

Methods: To determine the temporal transition of connective tissue degradation, the concentrations of urinary hydroxyproline (HYP), hydroxylsypyrindoline (HP cross-link), and lysylpyridinoline (LP cross-link) were assayed by reverse-phase high performance liquid chromatography (RP-HPLC) after prior acid hydrolysis, solid phase extraction and partition chromatography procedures according to previously published methods. Urinary creatinine (Cr) was also quantitated using a colorimetric assay to measure possible muscle rhabdomyolysis and to normalize the connective tissue bio-marker concentrations.

It would be unlikely to expect significant connective tissue changes within the short time frame that support severe decrements in bone structure and soft tissue remodeling. However, 13-21 days of weightlessness may have the potential of deconditioning soft tissue junctions, such as tendon and ligament junction interfaces (muscle and bone). The ligament junction interfaces may be weakened to the extent of creating a greater probability of soft tissue failure in situations that may require high strain rates, for example an emergency egress (escape from upper hatch). Limb suspension studies (2 or 3 weeks duration) have reported significant decrements in ligament junction strength. Also, the Bion program will give scientists the flight opportunity to begin modeling connective tissue metabolic profiles which can be used as an initial data base to compare with longer duration flight programs for moon-base, space station, and Mars missions. Furthermore, marker work can be used as first level non invasive analysis for determining effective countermeasures on Earth or in a microgravity environment that modify connective tissue metabolism (drugs, exercise, and combinations).

FY97 Publications, Presentations, and Other Accomplishments:

Kohles, S.S., Martinez, D.A., Bowers, J.R., Vailas, A.C., and Vanderby Jr., R. "Effect of a growth hormone treatment on bone orthotropic elasticity in dwarf rats." *Ann. Biomed. Eng.*, 25(1), 77-85 (1997).

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: ARC-21129

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 2

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9403056

Flight Assignment: Mir/Biorack

Responsible NASA Center: Ames Research Center

Task Description:

The unique environment of microgravity can place unusual stress on, and cause many physiological changes in, organisms that evolved in a 1-G environment. Some of the basic physiological changes include loss of fluids and electrolytes, muscle atrophy, space motion sickness, anemia, reduced immune response, and loss of calcium and mineralized bone. The bone loss that accompanies space flight is one of the most serious health hazards associated with, and impediment to, long-term manned missions. Biomedical studies of manned space flight have consistently indicated a continuous and progressive loss of calcium and weight-bearing skeletal bone. Several lines of evidence, from both human and animal studies, have demonstrated that the bone loss occurring in space flight is due to a decrease in bone formation. The decrease in bone formation and osteoblast growth is likely due to both direct and indirect effects of microgravity.

This flight research aims to analyze how microgravity affects bone loss by investigating alterations in select gene expression patterns. Biomedical studies show that humans and animals exposed to microgravity have continuous and progressive loss of calcium and weight-bearing skeletal bone due to lack of bone formation. The decrease in bone formation is related to the downregulation of gene activation of important growth regulatory elements in the cells. Previous studies have not been able to isolate ground effects from microgravity conditions. In this study, we will measure the activation of immediate early genes in quiescent bone osteoblasts by adding 10% fetal calf serum (FCS) media to quiescent cells to activate genes and cell growth in the presence of microgravity and onboard 1-G controls. Mechanical stress used as a countermeasure for bone loss has been demonstrated to cause release of prostaglandin E₂ (PGE₂) from osteoblasts. PGE₂ can increase trabecular bone formation in rats. The lack of PGE₂ synthesis occurring in space may be a critical factor responsible for the bone loss that occurs in astronauts. PGE₂ downregulation is likely a key component in the mechanism of bone

loss that occurs in astronauts. We will look at key genes responsible for osteoblast growth and homeostasis. These include the gene expression patterns of the elements responsible for PGE₂ synthesis and action: c-PLA₂ (cytosolic phospholipase A₂), COX-1 and COX-2 (cyclo-oxygenases), and the PGE₂ receptors EP₁, EP₂, and EP₃. Expression patterns will be analyzed in osteoblasts exposed to microgravity using rtPCR technology. These studies will identify the genes that are activated with and without gravity and will help us to determine the factors which regulate bone growth in space flight and which factors are directly due to microgravity.

Since the Fall of 1996, we have accomplished several milestones. First we were able to diagnosis major problems with the flight hardware before the January flight (STS-81). We passed an inspection by ESA on flight hardware readiness. Second we flew the osteoblasts successfully in January. The RNA from that flight is processed and the data now are being analyzed. Third, we have successfully completed the May flight experiments for STS-84. The RNA from that flight is processed and the data now are being analyzed. The RNA analysis is in its final stages and should be finished by late 1997 to early 1998. We will then publish data as rapidly as possible and post on our website <http://www.spacedu.com>.

The protein and coverslip data is not yet completed for all 3 flights and lab analysis will require at least another year for STS-76, 81, and 84 samples to be completed. In addition, baseline ground experiments for the flights are ongoing, with two peer reviewed papers published on the subjects in 1997.

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Asthma patients, Cushing patients, and astronauts that have osteoporosis have one thing in common: an increase in glucocorticoids. After analysis of Skylab data, it was reported that the glucocorticoids are increased on a daily average in astronauts. We followed up that discovery with studies on the ground where we used comparable amounts of glucocorticoids found in astronauts and patients and published data showing that the glucocorticoids decrease new bone growth by 50%. This growth is partially to fully reversed by addition of exogenous PGE₂. We have also found in our flight experiment on STS-56 that microgravity interferes with normal bone cell growth activation and causes reduced PGE₂ synthesis; this observation is in press in *Experimental Cell Research*. In recent studies, we have also noted that glucocorticoids reduce induction of early immediate genes by blocking the cyclo-oxygenase pathway. The effects can be reversed by addition of exogenous PGE₂. We are currently investigating the basic molecular mechanisms that control gene expression at the promoter region of the key oncogenes like fos and cyclo-oxygenase-2 that are needed for normal bone growth.

The lack of gravity in space flight also adds to the effects on bone loss since the necessary mechanical strain is missing in 0-G. Recent experiments have shown that mechanical strain of confluent osteoblasts results with a release of PGE₂ from the bone cells which is followed by elevated gene expression of cyclo-oxygenase needed for bone growth. This is probably the major mechanism by which exercise augments bone growth (manuscript in preparation).

Spin-off benefits: The new technology made possible by our NASA grant has allowed us to make headway in our studies of colorectal and prostate cancer. We have found that certain tumors (e.g., colorectal and prostate cancers) have altered expression of cyclo-oxygenase-2 which is a primary cause of unregulated growth in some of these tumors and may be the basis of aspirin protection from mortality in colorectal cancer patients.

FY97 Publications, Presentations, and Other Accomplishments:

Fitzgerald, J. and Hughes-Fulford, M. "Gravitational loading of a simulated launch alters mRNA expression in osteoblasts." *Exp. Cell Res.*, 228, 168-171 (1996).

Hughes-Fuldord, M. "Science for children day multimedia presentation." Marin County (June, 1997).

Hughes-Fulford, M. "Growth regulation by Cox-2 ." International Conference on Eicosanoids and Bioactive Lipids, San Diego, CA (September, 1997).

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Hughes-Fulford, M. "PG regulated growth." Berlin Free University (Winter, 1996).

Hughes-Fulford, M. "Space life sciences." San Francisco Rotary, San Francisco (Winter, 1996).

Hughes-Fulford, M. "Take your daughter to work day." UCSF, San Francisco (Winter, 1996).

Hughes-Fulford, M. "Three part educational environmental series video series "ecospeak" three videos: Series #1 Smog, Series #2 Ozone Depletion." Narration and Contributor in conjunction with the California Academy of Science (Summer, 1997).

Hughes-Fulford, M. "Growth regulation and gene expression in osteoblasts by prostaglandins." Proceedings of the International Conference on Eicosanoids and other Bioactive Lipids in Cancer, Inflammation and Radiation Injury, New York (1997).

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Lewis, M.L. and Hughes-Fulford, M. "Cellular responses to microgravity" in "Textbook for the International Space University." Edited by: Churchill, S. Harvard University, ch. 3 (1997).

Tjandrawinata, R. and Hughes-Fulford, M. "Upregulation of cyclooxygenase 2 by product, prostaglandin E2." Proceedings of the International Conference on Eicosanoids and other Bioactive Lipids in Cancer, Inflammation and Radiation Injury (1997).

Tjandrawinata, R., Dahiya, R., and Hughes-Fulford, M. "Induction of cyclooxygenase-2 mRNA by prostaglandin E2." *Brit. J. Cancer*, 75, (8), 1111-1118 (1997).

Tjandrwinata, R., Vincent, V., and Hughes-Fulford, M. "Vibrational force alters mRNA expression in osteoblasts." *FASEB J.*, 11, 493-497 (1997).

OsteoMass: Effects of Microgravity on Osteoblast Gene Expression in Variable Gravity

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-40-27-06
 Initial Funding Date: 1996
 Students Funded Under Research: 0
 FY 1997 Funding: \$325,000

Solicitation: 91-OSSA-15
 Expiration: 1998
 Post-Doctoral Associates: 2

Flight Information:

Flight Assignment: Mir/Biorack
 Responsible NASA Center: Ames Research Center

Task Description:

One of the most serious health hazards to long-term manned space flight is the loss of bone. This is the major physiological showstopper in a manned Mars mission. Biomedical studies of manned space flight have consistently shown a continuous and progressive loss of calcium and weight bearing bone. During the Skylab Missions, astronauts lost 4% of their bone over an 84 day period; the Soviet cosmonauts lost up to 19% of their bone during their long-term flights. Various lines of evidence from both humans and animal studies have demonstrated that the loss of bone in space flight is due to a decrease in bone formation and osteoblast growth. This loss of bone formation and osteoblast growth is probably due to both the direct and indirect effects of microgravity.

The objective of this research is to study both the direct and indirect roles which gravity plays in modulating the biological processes that regulates new bone growth. The first and direct effect of 0-G is the loss of natural mechanical stress experienced on Earth. Mechanical stress (exercise) has been used by both the Soviet and American programs as a countermeasure for bone loss in flight. Mechanical stress has recently been demonstrated to cause release of prostaglandin E₂ (PGE₂) from osteoblasts. PGE₂ has been shown to increase trabecular bone formation in rats and infants, but the mechanism of action of PGE₂ in stimulating osteoblast growth is unknown. A second and indirect effect of microgravity is an increase in cortisol in crew members. Urinary cortisol of crewmembers increased from an average preflight value of 54 + 4 µg/total volume to 94 + 5 in flight during the Skylab Missions. Glucocorticoid-induced osteoporosis has been noted in patients with Cushing's Syndrome and in patients treated with glucocorticoids for asthma and arthritis. Glucocorticoids are known to inhibit prostaglandin synthesis, and therefore, prostaglandins may play a pivotal role in the loss of bone in space and in disease states on Earth.

The growth and mineralization of osteoblasts is complicated and hard to simplify in the intact animal flown in space. In these ground-based studies, we will simulate the physiological conditions that change during space flight and therefore investigate the cell and molecular mechanisms that are associated with bone loss in 0-G. We

have developed a culture system using the MC3T3-E1 cloned osteoblast as our model to study the molecular mechanisms of bone formation. With this system, we have demonstrated that osteoblasts exposed to comparable concentrations of glucocorticoids observed during space flight have reduced prostaglandin and DNA synthesis and reduced growth. Our laboratory has demonstrated that addition of exogenous prostaglandin increases osteoblast growth and can overcome an indomethacin-inhibition of bone growth. We have new evidence showing that the prostaglandins alone can stimulate expression of the early growth oncogenes in the osteoblast.

Our first objective is to study the effect of mechanical stress on prostaglandin release and osteoblast cell growth. We will study the signal transduction of prostaglandin stimulated bone growth and will analyze the gene regulation of osteoblasts under inhibited and stimulated conditions. Our second objective is to understand the role of prostaglandins in bone loss. This information will help us understand glucocorticoid-induced bone loss both in space and in disease states. In all these objectives, we are using state-of-the-art methods of cell biology and molecular biology to help us understand the underlying mechanisms of signal transduction and stimulation of bone growth in the osteoblast. Studies of the basic mechanisms that regulate growth of bone cells in 0-G conditions could provide the preliminary information to establish medical intervention of bone loss, both in space and on Earth.

Since the Fall of 1996, we have accomplished several milestones. First we were able to diagnosis major problems with the flight hardware before the January flight (STS-81). We passed an inspection by ESA on flight hardware readiness. Second we flew the osteoblasts successfully in January. The RNA from that flight is processed and the data now are being analyzed. Third, we have successfully completed the May flight experiments for STS-84. The RNA from that flight is processed and the data now are being analyzed. The RNA analysis is in its final stages and should be finished by late 1997 to early 1998. We will then publish data as rapidly as possible and post on our website <http://www.spacedu.com>.

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Tjandrawinata, R., Vincent, V., and Hughes-Fulford, M. "Vibrational force alters mRNA expression in osteoblasts." *FASEB J.*, 11, 493-497 (1997).

Graviperception in Starch Deficient Plants in Biorack

Principal Investigator:

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available

Solicitation: 94-OLMSA-03

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$99,550

Flight Information:

Experiment ID: 9403063

Flight Assignment: Mir/Biorack

Responsible NASA Center: Ames Research Center

Task Description:

The purpose of this research is to study gravity perception in wild-type (WT) and starch-deficient mutants of the plant *Arabidopsis* in microgravity on the Biorack module aboard the space shuttle. This research involves two flight missions (STS-81 and STS-84). The specific goals presented in this proposal are: (1) to determine the optimal growth conditions of seedlings in the "lentil-roots" hardware on Biorack in ground-based testing; (2) to determine the threshold levels of stimulus required for gravitropic curvature in the microgravity-grown roots; (3) to study the distribution of integrin (a membrane protein which has a key role in signal transduction) in plant cells in ground-based studies; and (4) to determine if integrin localization is affected in plant cells from seedlings grown in a microgravity environment. This project is designed to investigate the starch-statolith model for gravity perception, a hypothesis which has been widely debated for the past century. We now have an opportunity to help resolve these controversies by using the unique characteristics of microgravity. Insights gained from this research should be applicable to other plant groups, including those that may be used during long-term space flight and/or International Space Station missions. The proposed work is directly related to the emphases of the NRA 94-OLMSA-03 since it is concerned with gravitational cell biology and plant biology, which are two of the four focal areas of this program. The STS-81 experiment flew in January 1997, and the STS-84 experiment flew in May 1997. Both were successful from an operational perspective, and we are actively analyzing the data in our laboratory.

PREPLASTID was a preliminary experiment on STS-81 (S/MM-05) which is designed to assess the growth characteristics of normal (wild-type, WT) *Arabidopsis* plants and three starch-deficient mutants. Based on the PREPLASTID results, the optimal development and stimulation times were selected for the larger PLASTID experiment on STS-84 (S/MM-06).

During the STS-81 flight, germination was greater than 90% for seeds in microgravity as well as the controls. Flight seedlings (F- μ g) were smaller compared to control plants grown on the ground and to control plants on a rotating clinostat. However, initial examination of the STS-84 results suggests that flight seedlings were

similar to those grown on a centrifuge in space (F-1g). Seedlings that developed in space had two structural features that distinguished them from the ground controls: a greater density of root hairs and an anomalous hypocotyl hook structure.

The slower growth and morphological changes observed in the flight seedlings may be due to the effects of ethylene in the space flight environment. These effects in the flight seedlings could be reproduced by adding exogenous ethylene (1 - 10 ppm) to seedlings grown on the ground in flight hardware.

Nevertheless, during the STS-81 flight, hypocotyls of WT seedlings responded to a unilateral 60-min stimulus provided by a 1-G centrifuge while those of the starch-deficient strains did not. (Curvature studies are in progress for the STS-84 flight.) Thus, the strain with the greatest amount of starch responded to the stimulus given in-flight, and, therefore, these data support the starch-statolith model for gravity sensing. This is the first space flight study to compare the response of a WT and mutant strains to a unilateral 1-G stimulus from an on-board centrifuge.

In our next set of space experiments on STS-84, we plan to examine the responses of these four strains of *Arabidopsis* to several different unilateral doses of gravity provided by the 1-G centrifuge. In these studies, data on both the root and hypocotyl should be available since a different camera position (in-flight) was used in order to better resolve curvature in the relatively small *Arabidopsis* roots. In addition, we also will further study the (likely) effects of ethylene on gravitropism of the seedlings.

Since we plan to study the structure of starch in microgravity-grown seedlings, our studies should aid in understanding basic starch structure and metabolism. Starch is the principal storage carbohydrate in plants and is an extremely important natural product in both agricultural and industrial settings. Starch is used extensively in foods and beverages, and can be converted to glucose and high fructose corn syrup. In terms of industrial applications, starch and modified starches are important in pharmaceuticals, detergents, paper products, coatings, resins, and numerous other products. For long-term space flight and the International Space Station, our research should aid in understanding how microgravity affects the development of starch and what implications this has for the food value of plants.

FY97 Publications, Presentations, and Other Accomplishments:

Guisinger, M.M., and Kiss, J.Z. "Gravitropism in plastid mutants of *Arabidopsis*." American Society of Plant Physiologists, Vancouver, B.C. Canada. *Plant Physiol.* 114(S):133 (1997).

Katembe, W.J., Swatzell, L.J., Makaroff, C.A., and Kiss, J.Z. "Immunolocalization of integrin-like proteins in *Arabidopsis* and *Chara*." *Physiologia Plantarum*, 99, 7-14 (1997).

Kiss, J.Z. "Gravitropism in the rhizoids of the alga *Chara*: A model system for microgravity research." *Biol. Bull.*, 192, 134-136 (1997).

Kiss, J.Z. "Plant development in microgravity: *Arabidopsis* on the U.S. Space Shuttle." Hungarian Plant Anatomy Symposium, Szeged, Hungary. Abstract Book, p. 35 (1997).

Kiss, J.Z. "The potential role of integrin-like proteins in plant gravitropism." European Low Gravity Association, Paris, France. Abstract Book, p. 30. (1997).

Kiss, J.Z., Guisinger, M.A., Miller, A.J., and Stackhouse, K.S. "Reduced gravitropism in hypocotyls of starch-deficient mutants of *Arabidopsis*." *Plant Cell Physiol.*, 38, 518-525 (1997).

Kiss, J.Z., Guisinger, M.M., and Miller, A.J. "What is the threshold amount of starch necessary for full gravitropic sensitivity?" *Advan. Space Res.* (In Press).

Kiss, J.Z., Katembe, W.J., and Edelmann, R.E. "Development of *Arabidopsis* seedlings in microgravity." American Society of Plant Physiologists, Vancouver, B.C. Canada. *Plant Physiol.* 114(S):133 (1997).

Kiss, J.Z., Katembe, W.J., and Edelmann, R.E. "Growth, development, and gravitropism of wild-type and starch-deficient mutants of *Arabidopsis* during spaceflight." American Society for Gravitational and Space Biology, Washington, DC. *ASGSB Bull.*, 11 (In Press).

MacCleery, S.A., and Kiss, J.Z. "Plastid sedimentation kinetics in roots of wild-type and starch-deficient mutants of *Arabidopsis*." American Society for Gravitational and Space Biology, Washington, DC. *ASGSB Bull.*, 11 (In Press).

Swatzell, L.J., Weise, S.E., Makaroff, C.A., and Kiss, J.Z. "Co-localization of integrin-like and α -actinin-like proteins in *Arabidopsis* columella cells." American Society of Plant Physiologists, Vancouver, B.C. Canada. *Plant Physiol.* 114(S):264 (1997).

Weise, S. and Kiss, J.Z. "Gravitropism of the inflorescence stems in starch-deficient mutants of *Arabidopsis*." American Society for Gravitational and Space Biology, Washington, DC. *ASGSB Bull.*, 11 (In Press).

Wood, P.C., Kiss, J.Z., and Makaroff, C.A. "Isolation and characterization of an integrin-like protein from *Arabidopsis*." American Society of Plant Physiologists, Vancouver, B.C. Canada. *Plant Physiol.* 114(S):264 (1997).

Mechanisms of Gravity Sensing and Response in Hematopoietic Cells

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Funding:

UPN/Project Identification: not available	Solicitation: 93-OLMSA-07
Initial Funding Date: 1995	Expiration: 1997
Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: \$0	
Joint Agency Participation: ESA	

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9307221
Flight Assignment: Mir/Biorack
Responsible NASA Center: Ames Research Center
Flight Hardware Required: Biorack Cytokines H/W

Task Description:

The overall objective of this proposal was to investigate the role of the cytoskeleton in gravity (microgravity) "sensing" and signal transduction in single cells (lymphocytes). Specific aims were to evaluate 1) cytoskeletal morphology; 2) signal transduction; and 3) expression of genes regulating cytoskeletal and related proteins and cytokines. Justification: Membrane-cytoskeletal interactions are involved in second messenger transduction by a signal amplification mechanism; intact microtubules are required. Our previous flight results showed altered actin morphology in mouse and *Xenopus* cells flown on STS-52 and 56, and HL 60 cells flown on STS-67 and STS-69. The effect of altered cytoskeletal morphology on signal transduction during space flight is unknown. The proteins gelsolin and profilin regulate actin polymerization and filament formation in cardiac myocytes. Function of these proteins is modulated by inositol diphosphate hydrolysis at the inner membrane. The effect of space flight on expression of genes for these regulatory proteins is not known.

First year activities to verify procedures and expand ground-based data were to: 1) develop primers to graduate mRNA gene transcripts by RT-PCR for the lymphocyte activation model proposed; 2) test cytoskeleton/PKC interactions by immunofluorescence to evaluate early signal transduction events; and 3) conduct an Experiment Sequence Test at NASA KSC to ensure cell survival and appropriateness of all procedures. Second year activities included flying the experiment and evaluating samples returned. Results proved a significant alteration of the microtubule cytoskeleton including coalescing of the filaments, disorganization of the microtubule organizing centers, and premature termination of the filaments. This was accompanied by retardation in cell growth in microgravity, increased glucose use, and an increase in programmed cell death (apoptosis). Samples were not evaluated by RT-PCR due to insufficient recovery of messenger RNA.

The most significant change in direction of this research after completion of the flight experiment and evaluation of the returned samples was toward investigation of the mechanisms involved in the observed increase in apoptosis in microgravity. Simulated launch perturbation experiments were conducted to define contribution to cytoskeletal anomalies and apoptosis. Collaboration with the ESA co-investigator was finalized and a series of experiments were planned for the next year to evaluate signal transduction by investigating different isoforms of protein kinase C (PKC) using samples from this experiment.

What has been accomplished: Alteration in cytoskeletal morphology in space flown lymphocytes appears to underlie mechanisms of gravity sensitivity through disruption of cytoskeletal and cell membrane integrity and signal transduction. Human T lymphoblastoid cells, Jurkat, were flown on the Biorack TCELL experiment to characterize microtubule organization, growth responsiveness, signal transduction, and apoptosis during space flight. Cell growth was stimulated in microgravity by increasing serum concentration. After 4 and 48 hours, cells were filtered from medium and fixed with formalin for evaluation of the cytoskeleton; at 4, 24, and 48 hours, cells were lysed with GITC for RNA analyses.

In 1996-97, characterization of the responses of the Jurkat cells flown in the TCELL experiment on STS-76 was finalized. Post-flight analysis by confocal microscopy revealed diffuse, shortened microtubule filaments extending from poorly defined organizing centers (MTOCs) in flown cells. In comparable ground controls, microtubules radiated in discrete filaments from organized MTOCs and branched toward the cell membrane. Flown cultures did not increase in number whereas ground populations increased by 40%. During flight, some cells continued cycle traverse as evidenced by presence of mitotic figures and DNA synthesis. Flown populations used significantly more glucose than ground controls. Samples were not evaluated by RT-PCR due to insufficient recovery of messenger RNA.

In 1997, research focused on further evaluation of TCELL samples for apoptosis. Presence of apoptotic bodies and time dependent increases in Fas/APO-1 protein in flown, but not ground samples, confirmed flight-related apoptosis. This observation raises a new question regarding spaceflight effects on cellular aging and programmed cell death in lymphocytes. Based on our observations, we conclude that cytoskeletal alteration, growth retardation, and metabolic changes in space flown lymphocytes are concomitant with increased apoptosis and elevated Fas/APO-1 protein and suggest that blunted growth response in lymphocytes during space flight is moderated by apoptosis. Our future work on this task, under a no-cost extension in 1998, will continue to evaluate the FAS and FAS-ligand mediated cell death in Jurkat cells and will evaluate the effects of launch vibration simulation on apoptosis and cytoskeletal morphology.

In addition to completion of the analyses above in 1997, the collaboration with ESA was activated. The PI spent eight weeks in the laboratory at Strasbourg, France working with co-investigator, Jason Hatton. The plan for further collaboration to expand the research in 1998 is focused on complementary analyses of samples available after completion of primary analyses of the PHORBOL and TCELL experiments from S/MM-03 and will involve analysis of cellular proteins as a way to determine certain gene responses and related mechanisms of apoptosis.

Information over the past twenty years of manned space flight consistently indicates a reduced response of human T lymphocytes to mitogenic challenge during, and for several days after, flight. Why this occurs has not been clearly defined. The number of inflight illnesses reported over the years indicates a high probability that susceptibility to illness can be a problem during long-term space flight. In 1992, Taylor et al., compiled data to confirm a significant inflight reduction in the cellular immune response of astronauts during space flight, and there is evidence that stress (Earth and/or space flight) and other factors, such as the increased growth rate of bacteria in microgravity, may increase the chance of infections. Thus, space flight-induced immuno-incompetence could present a serious problem for long-term human space exploration. On Earth, similar illnesses result from compromised immune cell function in immune deficiency diseases.

The information gained on the mechanisms involved in reduced human cellular immunity during space flight is applicable to space- and Earth-bound medicine and biotechnology since an understanding of mechanisms guides

drug design and countermeasures and has ramifications for treatment of immune system disorders including AIDS and cancer. The new direction, based on the results of TCELL on STS-76 in the area of cellular aging (apoptosis), can have impact on understanding of aging and reduced immune function in the elderly.

The two basic biological molecular level processes for which understanding can be gained from this research are the role of the cytoskeleton in gravity "sensing" and the mechanism by which gravity affects the expression of specific genes in the differentiation and growth of cells. We clearly demonstrated in experiments flown on STS-52 and STS-56 with mouse osteoblasts and *Xenopus* myocytes, that actin cytoskeletal morphology in flown cells is significantly altered compared to that of ground control cells held in the same hardware at the same temperature and using the same timeline as the flight experiment. We showed low-G effect on the cytoskeleton again STS-69 with HL-60 cells. The cytoskeleton does not polymerize elements to the same extent in flight as in ground cultured cells. Cells are also consistently growth retarded and utilize less glucose during space flight. Our results from the TCELL experiment show that Jurkat cells used twice as much glucose as ground controls. This may be a response to stress characteristic to lymphocytes since the previously flown osteoblasts used less glucose on a per cell basis. This observation illustrates the need for further space flight experiments to confirm the mechanisms causing differences in responsiveness in different types of cells.

This research investigates cytoskeletal assembly and function in an effort to learn how the cytoskeleton is involved in gravity "sensing." Information resulting from this research will advance the understanding of cell-level gravity "sensing" by defining the measurable effects on the cytoskeleton and related signal transduction pathways and specific growth and function regulation.

Short term problems encountered by humans in space flight have similarities to illnesses on Earth (such as osteoporosis, immune dysfunction, diseases of aging) for which no effective treatments have been developed (AIDS and some forms of cancer).

A better understanding of the regulatory processes of cell growth and differentiation, specifically lymphocytes, can facilitate rational design of drugs and development of therapeutic procedures for improving human health.

The survival of actively growing lymphocytes is controlled by cell surface receptors which specifically induce apoptosis. A member of the tumor necrosis factor family, the Fas protein, interacts with Fas ligand (Fas-L) to specifically induce cell death. An understanding of the role of this protein in cellular aging and death in microgravity may lead to a better understanding of immune function in our aging population.

Potential for production of cytokines in low-G and potential for development of new drugs based on information gained from this research are significant benefits from this research.

FY97 Publications, Presentations, and Other Accomplishments:

Lewis, M.L. "Gravity sensing in human lymphocytes (Jurkat) involving the microtubule cytoskeleton." (Abstract) ASGSB Bull., 10:447 (1996).

Modification of Radiogenic Damage by Microgravity

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Funding:

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Flight Information:

Experiment ID: 9307320

Flight Assignment: Mir/Biorack

Responsible NASA Center: Ames Research Center

Task Description:

The goal of the proposed experiment is to measure the dose versus response relationships for radiation-induced mutation and chromosome aberration *in vivo* in an animal in the presence and absence of gravity to determine whether gravity unloading results in dose modification. If dose modification occurs, it means that risk assessments for astronauts exposed to radiation in space may need to be revised and/or that spacecraft shielding designs may need to be modified to accommodate potential reduced or enhanced radiosensitivity. Complementing this experiment is a second study aimed at isolating and determining the molecular structures of mutations induced in two structural genes by ambient space radiation.

The first year's work will involve development of an inflight irradiator based on a Strontium-90 radioisotope source and calibration of dose versus response relationships for existing mutation and chromosome aberration assays to this type of radiation in containers equivalent to flight hardware. The nematode *C. elegans* is used to measure forward autosomal recessive lethal mutation using a balancer chromosome technique. Stable anaphase bridges in intestinal cells are scored histologically to quantify chromosome aberration. Mutations in the genes *unc-22* and *fem-3* are isolated from worms exposed to the ambient radiation environment and characterized for

their molecular structures. Physical dosimetry is provided by thermoluminescent and plastic nuclear track detectors.

Worms are exposed to 0- or 1-G in experiment unique hardware interfaced to the Type I containers of the ESA Biorack with its centrifuges and incubators providing the appropriate environments. Radiation exposure is accomplished by transferring dormant worm larvae to containers preloaded with different activities of the Beta particle emitting isotope calcium-45.

The results of the experiment indicated that gravity did not act as a dose modifying factor with respect to radiation-induced mutation. Technical difficulties reduced sample yields for aberration experiments which are currently undergoing further statistical assessment. Twenty-five mutations in the *fem-3* gene but zero in the *unc-22* gene were isolated. *fem-3* mutants are still undergoing molecular analysis. Continuing into year two, these calibrations will measure effects of temperature variation and timing to provide a measure of variance under operational conditions so the detection limits for differences due to gravity can be established in flight.

This proposal was submitted 4/25/94 in response to NRA 93-OLMSA-07 with the purpose of testing the hypothesis that microgravity can alter the physiology of a small organism, *C. elegans*, in such a way as to modify its ability to repair radiation-induced damage vis-a-vis unit gravity conditions. The results of this study are important in assessing, in principle, whether estimates of human health risks from exposure to space radiation must be amended to include gravity as a dose modification factor.

The original proposal set forth a plan to measure mutation and chromosome aberration in nematodes exposed to Beta particle radiation from a radioisotope source sealed in a shielded chamber. The exposure conditions were to have been comparable to laboratory experimental conditions for which extensive measurements are available. Specimens were to have been exposed as newly hatched larvae for chromosome aberration measurements or as young adult hermaphrodites for mutation such that the target cells for radiation-induced damage would be gametes in various stages of meiosis.

Following peer review and a science working group between NASA and ESA, the proposal was approved in a rescoped form for implementation on STS-76 using the ESA Biorack facility. While the science objectives remained the same, the protocols and hardware configuration were modified in order to conduct the experiment within the constraints of the mission resources and Biorack interface. The technical solution was to transfer nematodes in orbit to culture containers preloaded with the unsealed radioisotope calcium-45. Subsequent to the rescoped plan, ESA/NASA resources changed again and a final readjustment in scope was agreed to in which additional Biorack resources became available and subcontracts to U. of San Francisco (USF, Dr. Eugene Benton) and Texas Christian University (TCU, Dr. Philip Hartman) were let to acquire additional data on mutation due to natural space radiation and to provide supporting physical dosimetry measurements.

The experiment was successfully flown on STS-76 and samples were returned to KSC Hanger L where they were prepared for analysis of induced mutation and chromosome aberrations at JPL, ambient radiation-induced mutations at TCU, and physical dosimetry at USF. At the time of the mission, a series of additional ground control experiments remained to be performed in support of the flight experiment series.

Mission Operations

The STS-76 or S/MM-03 (Biorack short title "Elegans") was performed aboard the Spacehab / Biorack facilities with two deviations from planned operations. First, a one day launch delay occurred because of weather in Florida. Second, the termination of the mission was altered due to mission replanning and weather in Florida. This ultimately resulted in exposure of biological specimens undergoing mutagenesis to an unplanned gravity level profile during the final 48 hours of the mission. On orbit procedures were performed satisfactorily by the crew with some expansion of the planned timeline that was inconsequential.

Ground control operations were performed in parallel to the flight operations. It is likely that some sedimentation occurred in ground control samples during nematode syringe operations that resulted in a reduction

in sample size for some cultures. In hindsight, supplementary resuspension steps should probably have been added to the ground control procedures rather than following the flight procedures precisely. Significant sample recovery difficulties were encountered post flight in the chromosome aberration experiments resulting in a reduction in sample size. The problem was traced to lot differences in plastic pipettes which led to nonspecific absorption of worms.

Biorack Performance

Temperature and centrifuge profiles for the Biorack 220C incubator indicate that the facility performed properly and within desired limits during operation. Biorack support equipment such as passive thermal control units and ambient stowage lockers also performed properly resulting in proper incubation conditions for nematodes. The samples frozen in the LSLE freezer were returned to KSC in an appropriate frozen state.

Experiment Unique Equipment (EUE) Performance

JPL-built EUE performed within acceptable limits for transfers of animals between source culture tubes and isotope-containing sample tubes. Slight back pressure in some syringes resulted in minor droplet formation which was contained by wiping needle tips on filter paper blotters and by returning the syringes post injection to their double tube assembly. Post flight wipe tests showed that minor levels of radioactivity were present in droplets associated with shielding structures but there was no evidence of contamination outside EUE. Ambient exposure tubes performed nominally with respect to mechanical integrity and containment but some biological samples showed poor viability. This reduced viability was traceable to culture preparation conditions.

Results

⁴⁵Ca Beta Particle Mutagenesis

The ⁴⁵Ca mutagenesis screen utilizing nematode strain JP10 was successfully completed. Irradiated dauer larvae parental (P0 generation) worms were washed free of ⁴⁵Ca at Hanger L and shipped back to JPL for analysis. Recovery of dauers was normal and P0 worms were dispensed to separate culture containers where they reproduced. Progeny (F1) worms were isolated and analyzed for the presence of autosomal lethal mutations in a 1500 gene region balanced by reciprocal translocation eT1 (III;V).

Four dose versus response curves have been determined: Flight 0-G & 1-G and ground 1-G & 1.4G. These curves represent the fraction of F1 animals bearing at least one lethal mutation in the balanced region as a function of accumulated dose. Note that each individual measurement represents both a different dose and dose rate condition. The data from this experiment appear to indicate that there is no substantial effect of gravity on the yield or level of expression of mutations induced by the Beta particles.

Analysis of F1 sterility is a measure of radiation-induced dominant lethality and physiological damage to gametes. In irradiated adult animals, this parameter is dose dependent but in irradiated dauer larvae, damaged germ cells apparently do not mature or are destroyed. Thus, the sterility vs. dose relationship under the flight protocols is useful as a corroborative measurement but is required for bookkeeping and records the variability in gamete viability.

A variable number of available wild type JP10 P0s was observed following recovery of dauer larvae from flight sample tubes, each of which nominally contained 1000 worms. To provide for 1000 F1s per dose level 110 Wild Type P0s were sought per tube and were picked to individual culture dishes. Extra P0s were not used. It was often observed, especially in ground samples, that less than 110 P0s were available, in which case all available worms were picked and extra F1s per P0 were used to augment the F1 sample size for mutation screening. We speculate that sedimentation of worms over time while in the syringes may have led to a reduction in the numbers transferred. As six samples were inoculated per syringe volume, this predicts that the number of available P0s should decrease monotonically with the sequence of tubes injected in each group of six

samples. Counts of total POs verifies this prediction. A lesson learned here is that the ground procedure should not necessarily exactly duplicate the in-flight procedure. In the case of the "Eelegans" experiment a gentle agitation step should have been included between injection steps in ground controls to resuspend the worms.

45Ca Beta Particle Aberration Induction

Frozen samples of worms derived from irradiated first stage larvae were washed free of 45Ca at Hanger L by a combination of centrifugation and filtration procedures. Initial examination of samples indicated a smaller sample size of adult worms than expected in the sample tubes but an adequate number for analysis. Monitoring of worms through the wash steps also indicated a significant reduction in sample size due to nonspecific binding of specimens to plastic pipettes. Thus a remedial recovery procedure was performed to supplement the main sample sets. Washed worms were fixed in Carnoy fixative and placed on ovalbumin - subbed microscope slides where they were air dried and shipped to JPL for histological analysis. Microscopic observation of these slides indicated that a majority of the worms in these samples were lost during the washing steps. Additional ground controls were performed to assess the variation in the dose versus response so that the measured reactions of the sparse flight samples could be assessed by statistical methods.

Ambient Radiation Mutagenesis

The Texas Christian University group has examined wild type and fem-3 worms from ambient exposure tubes. They have screened dauer larvae for the presence of unc-22 mutations and fem-3 loss of function mutations. Surprisingly, no unc-22 mutants were detected; this is in contrast to the unc-22 mutant screen conducted as part of a nematode experiment on IML-1 in which 13 unc-22 mutants were obtained in flight or similar radiation environment and exposure. However, 25 fem-3 frequency of 2.07×10^{-5} is 3.3 fold greater than spontaneous rates of 6.3×10^{-6} . The frequency of fems and enhancement over control rates is quite similar to the IML-1 unc-22 results. The fem-3 mutant provide an excellent sample set for detection of a space radiation mutant structure spectrum following molecular analysis. Southern hybridization analysis of these mutants is nearly complete.

Viability was an issue in both wild type and fem-3 dauer cultures. A significant fraction of ambient exposure culture tubes were subnormal in viability. Large lot differences were found indicating that properties of the dauer cultures themselves resulted in the different viability levels. The most likely explanation is that trace media components which dauer larvae perceive as food signals antagonized the estivation-promoting pheromone signal that dauers generate. It is unlikely that biocompatibility with hardware was the problem as the tubes were from the same lot as used with no incident on IML-1 (STS-42).

Physical Dosimetry

CR-39 plastic nuclear track detectors and lithium fluoride thermoluminescence detectors (TLD) were unloaded from Biorack Type 1 containers and shipped to University of San Francisco for analysis. The CR-39 material, which is perishable, was immediately processed by track etching and archived. The TLDs have been annealed and photometric data have been acquired. The data on total dose and dose composition of charged particles indicates that the ambient radiation environment was typical for a high inclination shuttle flight and similar enough to the STS-42 environment for nearly direct comparisons.

Summary

The STS-76 Biorack "Eelegans" experiment was successfully flown and flight specimens have been processed for data analysis. Results indicate that hardware performance and mission operations were satisfactory. Experimental results suggest no large differences in induced mutation frequency vs. dose responses as a function of gravity level. Ambient exposure samples have yielded fem-3 mutants but not unc-22 mutants. These fem-3 mutants are nearing completion of molecular analysis. Recovery of chromosome aberration specimens was disappointing due to nonspecific absorption to liquid transfer containers. A statistical evaluation is still in progress to compare flight data to an expanded ground control series. Physical dosimetry has been completed and

indicates a typical high inclination shuttle orbit radiation. Technical work was temporarily suspended during the transfer of the PI from JPL to Loma Linda University. A final report is in preparation and will be submitted at the earliest practical date. Final oral presentation of the experimental results is expected for the Biorack Shuttle to Mir symposium scheduled for June 1998.

This study attempts to determine whether there is a cellular or physiological response to altered gravity levels which may contribute to an organism's response to another environmental stress — exposure to radiation. Such a response would be a basic biological process. However, if it were determined that such a regulatory process existed, then it might be possible to manipulate the response. The capacity to manipulate the regulatory response would almost certainly be of clinical value by providing a means to alter radiation responses between normal and malignant tissues thus improving the prospects for radiotherapy.

FY97 Publications, Presentations, and Other Accomplishments:

Kazarians, G., Kern, R., Nelson, G., and Schubert, W. "Modulation of radiation-induced genetic damage by microgravity: Results from space shuttle STS-76 experiment." 45th Annual meeting of the Radiation Research Society, Providence, Rhode Island (May 3 - 7, 1997).

Nelson, G., Kazarians, G., Schubert, W., Kern, R., Schranck, D., Hartman, P., Hlavacek, A., Wilde, H., Lewicki, D., Benton, E.V., and Benton, E.R. "Radiation effects in space: Results from the STS-76 space shuttle mission." Abstract 439. International *C. elegans* meeting, Madison Wisconsin (June 2 - 6, 1997).

Bacterial Growth on Surfaces in Microgravity and on Earth

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Funding:

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Students Funded Under Research: 2
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Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9403044
Flight Assignment: Mir/Biorack
Responsible NASA Center: Ames Research Center
Flight Hardware Required: ESTEC

Task Description:

In the context of human life support in space flight, there is clearly a need for the highest possible bacterial water quality to limit the risks of infections in human occupants and minimize water system deterioration. Biofouling bacteria such as *Burkholderia (Pseudomonas) cepacia* are among the most common organisms isolated from space shuttle water systems. We have developed approaches on Earth which are useful for investigating biofilms in the spacecraft environment. We propose to determine the effects of space flight and microgravity on the formation of biofilms by bacteria. Procedures for preparation and storage of bacterial cells, growth media, physiological indicators, and fixation will be developed and evaluated. We will also evaluate techniques to be used to examine post-flight samples, including physiological assays and physical methods such as scanning confocal laser microscopy with image analysis. Our overall goal will be to establish experimental protocols for Biorack experiments to determine the effects of space flight and microgravity on biofilm formation by water-borne bacteria, and their control. The information obtained will improve our understanding of bacterial biofilms and their control both in spacecraft and on Earth. The data obtained may be used in the design of biological waste treatment systems for future use in spacecraft. It will also be important in the development of microbial systems for the commercial production of novel compounds in microgravity.

In preparation for the flight experiment, a vibration and centrifugation test was performed at NASA Ames Research Center in December, 1996. These tests were intended to assess both the reliability of the flight hardware and the effects of simulated launch conditions on the bacteria. The hardware was found to withstand all of the simulated conditions. There appeared to be some effects of vibration and centrifugation, both separately and together, on the growth of the microorganisms, compared with those that were not exposed to these treatments. Vibration and centrifugation enhanced the growth of *B. cepacia* in water, nutrient medium, and iodine solution. This experiment will be repeated with more detail in FY98.

The flight experiment was performed on Shuttle Atlantis flight STS-84, which was launched January 12, 1997 and returned on January 22. The results indicate that biofilms were formed both in all environmental conditions, viz., microgravity, 1-G centrifuge on board, Earth 1-G centrifuge on Earth, and Earth gravity. Bacterial growth was enhanced in microgravity, particularly in iodine solution and the associated biofilm. Data analysis and interpretation will be completed in FY98.

Data from the flight experiment (January 1997) suggests that the biofilms grew about as well or slightly more rapidly in microgravity compared to the space flight centrifuge 1-G and Earth-based controls. This suggests that water treatment on inhabited spacecraft may be more difficult than Earth-based experiments have suggested because bacteria may grow more rapidly and survive disinfection with iodine. Thus, because it has been shown that bacteria can associate with surfaces and form biofilms in microgravity, it should be possible to utilize surface-associated, immobilized bacterial cultures for the production of specific novel compounds in spacecraft. This will be of benefit in some industrial applications of space flight.

FY97 Publications, Presentations, and Other Accomplishments:

Pyle, B.H., Lisle, J.T., Broadaway, S.C., and McFeters, G.A. "Rapid detection of bacteria in spacecraft water systems." Society for Automotive Engineers Technical Paper Series No. 972421. Presented at the 27th International Conference on Environmental Systems, Lake Tahoe, NV (July, 1997).

Gravitropism and Autotropism in Cress Roots

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$76,000

Joint Agency Participation: ESA

Flight Information:

Experiment ID: 9403023

Flight Assignment: Mir/Biorack

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Biorack, photobox

Task Description:

The phenomenon of autotropic growth in cress roots has been observed in previous experiments under microgravity. We propose to investigate and quantify the interrelation between gravitropic curvature and autotropic straightening. By lateral stimulation, different gravitational stimuli - varying stimulus intensity and stimulation time - will be applied to cress seedlings (*Lepidium sativum*). Flight centrifugation will apply μ -G, 0.1-G, and 1-G for 3-60 minutes to roots that have been germinated for 26 hours. Under microgravity conditions, the long-term behavior of roots following actual G-induced curvature will be documented using time-lapse image capture.

Root growth analysis should enable us to determine the relative strengths of response to a limited dose (g -stimulus) following withdrawal of that stimulus, and some "memorized" previous angle of equilibrium growth. It will also be determined whether autotropic straightening results only from new growth (in which case a record of older curvature should be maintained), or whether regions that curved previously later straighten. It will also be possible to determine whether the extent of straightening is affected by the intensity of the previous lateral centrifugation. Differential growth occurs through changes in the shape of cells (length to width ratios or form factors). The form factor of cells in curving and straightening regions will be determined to establish the basis for the tropic response. This will be correlated with changes in the distribution of various cytoskeletal components (F-actin, α - and β -tubulin, rho, myosin, profilin, etc) both in the loci of curvature and in the rootcap. Differences between ground and microgravity controls will help establish the extent of interaction between G-forces, cytoskeletal proteins, and organelles such as amyloplasts.

Our objective is to characterize the nature of autotropic straightening in roots of *Lepidium* (cress) in both a space flight experiment and on the ground using a clinostat. A previous flight experiment used lateral centrifugation

to induce gravitropic curvature in cress roots. When these roots were then transferred back to microgravity, they were seen to straighten (i.e., there was a loss of gravitropic curvature). However, initial ground-based studies did not show such autotropic straightening when gravitropically curved cress roots were rotated on a clinostat. Therefore, we investigated whether autotropism occurred on a clinostat.

Since the term autotropism has been used to cover many phenomena, we critically reviewed the subject (Stankovic et al., in press). We suggested that the term "autotropism" should not be applied to the phenomenon of organ straightening that occurs *during* the course of gravitropism, since this straightening is part of a complex series of local growth adjustments overall through time, and since this phenomenon is not itself a tropistic response to a directional exogenous stimulus. Instead, we argued that the term autotropism should be used only for the phenomenon of organ straightening that occurs *after* the g-vector is randomized on a clinostat or withdrawn in the microgravity conditions of space flight

Next we determined that autotropic straightening of cress roots does occur on a clinostat. Roots that were horizontal for 1 h and 5 h curved down 63 and 90 degrees, respectively. After 6 h of clinostat rotation, gravitropic curvature was lost from almost all roots and they straightened. Straightening resulted from the angle of new root growth on the clinostat moving closer to the prestimulus vertical as well as from the loss of gravitropic curvature in older regions. On average, roots straightened about 38 degrees on a clinostat regardless of the length of previous horizontal stimulation. Because the final root angles after rotation were 24 degrees (1 h horizontal) and 53 degrees (5 h horizontal) from the prestimulus vertical, both sets of roots apparently retained some gravitropism. Control roots (moved from the vertical to the clinostat without horizontal stimulation) were slanted at various angles after 6 h of rotation. These results provide a direct demonstration of autotropic straightening of gravitropically-curved roots after the withdrawal of the directionality of the g stimulus through clinostat rotation. They indicate that gravitropic curvature is not necessarily permanent, and that the root retains some commitment to its equilibrium orientation prior to gravitropic stimulation.

In January, 1997 we conducted a space flight experiment on Biorack. Eight cuvettes containing cress seeds were used. Roots were either transiently exposed inflight to g-stimuli of varying intensities and then returned to microgravity or kept in microgravity throughout. Some roots were fixed for microscope-based examination on the ground and the behavior of other roots was followed in microgravity using time-lapse photography. Initial observations suggest that these roots straighten autotropically as well. Data to be gathered include determination of reciprocity ($g \times \text{time}$) in straightening, the kinetics of straightening in comparison with ground controls, the locus of straightening, and a comparison of post-stimulation scenarios with models predicting random root behavior.

This research is in fundamental plant root biology and does not address disease or therapeutics, nor is it likely to have any foreseeable direct impact on humans or in new technologies. It does, however, address basic biological questions of widespread interest, i.e. "how do plants' roots grow down?" The basic question is whether there is some sort of "memory" in the root gravitational response following conflicting and successive reorientations or g-excursions. We are also addressing questions about threshold effects that can only be answered in space. It is conceivable that this information will be valuable in optimizing the growth of crops for prolonged flight missions with humans.

FY97 Publications, Presentations, and Other Accomplishments:

Stankovic, B. and Sack, F.D. "Autotropism, automorphogenesis and gravity." *Physiologia Plantarum* (In Press).

Stankovic, B., Volkmann, D., and Sack, F.D. "Autotropic straightening after gravitropic curvature of *Lepidium* roots." *Plant Physiol., Suppl: Abstract 620* (1997).

Stankovic, B., Volkmann, D., and Sack, F.D. "Autotropism automorphogenesis, and gravity." *Plant Physiol., Suppl: Abstract 622* (1997).

Stankovic, B., Wolverton, C., and Sack, F.D. "*Arabidopsis* promotor trap lines with root tip and cap specific patterns of gusA expression." *Plant Physiol.* 114, Suppl: Abstract 1622 (1997).

Effects of Microgravity on Lymphocyte Activation: Cell-Cell Interaction and Signaling

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Students Funded Under Research: 0

Post-Doctoral Associates: 3

FY 1997 Funding: not available

Flight Information:

Experiment ID: 9403079

Flight Assignment: Mir/Biorack

Responsible NASA Center: Ames Research Center

Task Description:

Lymphocyte activation involves a complex sequence of molecular events, including intercellular and intracellular signaling. A number of studies, both during space flight and on the ground, show *in vitro* lymphocyte activation is inhibited in altered gravity conditions. The majority of these experiments have assessed activation based on radiolabeled thymidine incorporation at 72 hours post-activation as a marker of DNA synthesis. However, a temporal and mechanistic understanding of the inhibition is still lacking. Our laboratory has investigated the inhibition of lymphocyte activation under simulated hypogravity conditions (clinostat) by examining several temporal and functional points along the activation pathway. We have found that activation of lymphocytes by mitogenic lectins or antibodies exhibits a block very early at the transition from the G0 to the G1 stage of the cell cycle. If phorbol ester and calcium ionophore pairs are used for activation, this G0/G1 block is passed, but a later block at or near the G1/S cell cycle transition is noted.

Our hypothesis is that hypogravity inhibits lymphocyte activation by altering cellular signaling required for activation. This effect results from changes in intercellular biophysical interactions. Attempts to understand lymphocyte inhibition in microgravity must, therefore, consider both intercellular monocyte-lymphocyte interactions as well as the resulting intercellular signaling events. Specific tests described in this proposal will include the microscopic measure of 1) the formation of intercellular signaling complexes; and 2) cytoskeletal transitions in response to these events. These experiments will provide insights into the potential biophysical mechanisms involved in the inhibitory effects of gravity on cells and tissues.

This experiment was successfully performed on STS-81 and STS 84. The experiment preparation procedures were completed nominally at KSC. Three different methods of T-cell activation were performed successfully in-flight: (i) TCR mediated activation of T-cells using anti-TCR mAb to activate T-cells in a accessory

cell-dependent fashion; (ii) Immobilized anti-TCR on the surface of cell-sized beads to induce an activation signal in purified T-cells without the involvement of other cell types; and (iii) Direct activation of intracellular pathways with PDB/I to bypass receptor engagement and aggregation requirements for activation.

Minor modifications to the flight hardware were required between flight increments based upon observation of STS 81 inflight video and crew comments. In addition, STS 84 also had an altered timeline for the experiment compared to STS 81. The fixation time was extended to fit within the constraints of the mission timeline. This required a reduction in the concentration of the fixative to minimize the likelihood of overfixation of the samples. The fixation protocol was developed based on ground-based simulations with different fixation times.

Samples were retrieved at KSC within hours of shuttle landing and returned to JSC for processing and analysis. Approximately 60% of the sample analysis has already been completed for this experiment and the remaining analysis is currently underway. The surface expression of activation markers CD69 and CD25 was measured by post-flight immunofluorescence staining and flow cytometry analysis. Total intracellular F-actin was measured by post-flight permeabilization of cells and fluorescence labeling of F-actin with FITC-Phalloidin. Post-flight preparation of slides for immunofluorescence microscopy has been performed. Scanning Confocal Microscopy is currently being used to obtain optical cross section and 3-D optical reconstruction of the tubulin organization inside the cells. Extensive analysis of samples is currently underway to characterize the polarization of the cytoskeletal system in T-cells activated with bead-bearing antibodies in microgravity and in 1-G. Samples have also been preserved in appropriate fixative solution for analysis by SEM. Analysis of these samples will be performed to determine the overall surface morphology of the T-cells.

The Cell Biology Discipline Working Group has identified several high priority areas for investigation under the Space Biology Program. These include signal transduction systems, cell-cell interaction, and cytoskeletal structure. The experiments in this investigation will include elements of each of these research areas and will improve the understanding of environmental and gravitational influences on cells in culture.

The elucidation of factors regulating entry and progression through the cell cycle is currently of extreme interest to oncology, immunology, and developmental biology. Recently, a number of disciplines have converged in establishing a model for cell cycle regulation that accommodates the deregulated cell division in cancer, cell cycle delay in response to radiation, and the eventual failure and arrest of the cell cycle during aging. The system of hypogravity-mediated cell cycle arrest provides a unique experimental system to examine the tenets of this regulatory model. The ability to uncouple signal transduction systems through the use of hypogravity culture without the use of chemical agents or metabolic poisons provides the unique potential to investigate the details of these integrated elements in a more natural or physiological state.

Microgravity and Signal Transduction Pathways in Sperm

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Funding:

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FY 1997 Funding: \$175,000

Flight Information:

Experiment ID: 9403057
Flight Assignment: Mir/Biorack
Responsible NASA Center: Ames Research Center

Task Description:

The overall goal of the project is to determine whether second messenger signal transduction pathways in sperm are altered in microgravity compared to Earth-normal gravity. A previous experiment which examined a limited number of sperm movement parameters demonstrated that bovine sperm motility is altered significantly in microgravity. Prior to that study, exposure of sperm to hypergravity was found to produce a dramatic decline in the content of ATP and a rise in ADP in sperm; motility in these experiments was not determined. In this connection, sperm able to swim against a 1-G force were found to contain higher levels of ATP. Since ATP is critical to sperm motility, it is likely that microgravity may produce changes in sperm motility. Sperm motility is regulated by the content of cAMP, calcium ions, and the state of protein phosphorylation modulated by these second messengers. Whether these components are altered during changes in gravitational forces is not known. The present study will examine changes in protein phosphorylation in sea urchin under a variety of physiological and gravitational conditions, including those which promote oocyte fertilization on the ground in the presence and absence of speract, a chemotactic peptide from sea urchin eggs. We will also examine if protein phosphorylation in sperm activated in microgravity in sea water made with deuterium oxide (heavy water) is similar to activation at 1-G in normal sea water. The project will also yield information regarding biochemical mechanisms underlying the alterations in movement produced by alterations in gravity. Results from these experiments will greatly expand previous data regarding the effects of altered gravity on sperm function and early fertilization events. The flight experiments for this project will be carried out on Shuttle/MIR docking flights STS-81 (S/MM-05) and STS-84 (S/MM-06).

All of the planned flight experiments were successfully conducted on STS-81 and STS-84. Ground controls were successfully completed as planned 2 hours after the flight experiments. All samples were received at the University of Kansas Medical Center and sample analysis is under way. The hypothesis that microgravity has an effect upon protein phosphorylation during initiation of sperm movement was supported in both flight experiments. In both cases, phosphorylation of a 32 and 29 kDa pair of phosphoserine-containing proteins, and

a 130 kDa phosphothreonine containing protein showed significantly higher rates of phosphorylation in microgravity as compared to 1-G controls. Furthermore, the peak of phosphorylation of these proteins occurred earlier in microgravity than at 1-G suggesting that the duration of optimal sperm function might be shortened in microgravity. Additional analysis of these proteins demonstrated that these three proteins are components of flagella and may be subunits of outer arm and inner arm dyneins. The hypothesis that the effect of microgravity on sperm motility could be due to lower fluid viscosity was tested by activating sperm in artificial sea water made with either heavy water (D₂O) or regular water (H₂O). In both microgravity and 1-G, heavy water diminished the rate of phosphorylation of the flagellar phosphoproteins when compared to the respective H₂O controls. However, the pattern of inhibition by heavy water was different in microgravity than at 1-G, suggesting that viscosity alone does not fully explain the microgravity effect upon sperm. The third hypothesis, namely that sperm may respond differently to chemotactic egg peptides in microgravity compared to 1-G, awaits analysis of the samples that were prepared on STS-84. These results demonstrate that microgravity exerts its effect upon sperm motility via a direct effect upon the flagellum and includes alterations in subunits that appear to regulate utilization of ATP by the flagellum. Key questions raised by these results are 1) whether the efficiency of fertilization of sperm is altered in microgravity, and 2) is motility also altered in microgravity in the sperm samples that show a increased phosphorylation.

This study represents an important breakthrough in the general field of male aspects of reproductive biology as well as the cell biology of other cells and tissues that utilize motor proteins in flagella and cilia to generate movement. Such cells include sperm, the inside lining of the female reproductive tract involved in egg transport, some species of algae, and parts of the mammalian nervous system. Impaired sperm motility is a significant lesion in certain cases of male infertility. With regard to sperm, these results offer some specific cellular components that might be useful targets of analysis in such infertility cases. In addition, our analysis of the STS-81 and STS-84 flight and ground data has demonstrated that the rate of phosphorylation of these proteins is greater in microgravity than at 1-G. Such results suggest either that the efficiency of fertilization (i.e., percentage of eggs fertilized) in microgravity could be reduced if the window of sperm capacity to fertilize is shortened, or the converse could be observed if the functional result is that the sperm can get to the egg faster. Future flight experiments using both sea urchin sperm and eggs have been proposed to address these later questions.

In order to investigate signal transduction during initiation of sperm motility in microgravity, simpler methods for activation and fixation of sperm for subsequent post-flight analysis were developed. In addition, a new method for collection of echinoderm sperm that yields completely immotile sperm that are stable for up to 96 hr at 5°C and up to 24 hr at 22°C without becoming motile was also developed. Prior methods of analysis suggested that phosphoserine-containing proteins in the 32-29 kDa range were important for regulation of motility in diverse species from mammals to sea urchins. However, these proteins were usually buried in a very high background of other proteins that were apparently not related to motility. Our studies showed that the 32-29 kDa proteins as well as a 130 kDa phosphothreonine-containing protein are key components of the 'ignition system' of the sperm tail. In addition, several new phosphothreonine-containing proteins that are related to the initiation of motility have been identified. This conclusion has been drawn because the new preparation method prevents the background phosphorylation and motility that occurred in the older methods.

FY97 Publications, Presentations, and Other Accomplishments:

Bracho, G.E., Fritch, J.J., and Tash, J.S. "A method for preparation, storage and activation of large populations of immotile sea urchin sperm." *Biochem. Biophys. Res. Commun.*, 237, 59-62 (1997).

Bracho, G.E., Fritch, J.J., and Tash, J.S. "Identification of flagellar proteins that initiate the activation of sperm motility *in vivo*." (Abstract) *J. Cell Biol.* (In Press).

Bracho, G.E., Fritch, J.J., and Tash, J.S. "Inhibition of sperm motility and dynein ATP-ase activity by cAMP-dependent protein kinase inhibitors." (Abstract) *J. Cell Biol.* (In Press).

Bracho, G.E., Fritch, J.J., Landis, M.E., and Tash, J.S. "Heavy water inhibits changes in protein phosphorylation during initiation of motility in sea urchin sperm." (Abstract) ASGSB Bull., 11, 72 (1997).

Tash, J.S., Fritch, J.J., and Bracho, G.E. "Identification of flagellar protein phosphorylations that initiate the activation of sperm motility *in vivo*." (Abstract) Biol. Reprod., 56, 230 (1997).

Tash, J.S., Fritch, J.J., Landis, M.E., and Bracho, G.E. "Microgravity stimulates changes in protein phosphorylation during initiation of motility in sea urchin sperm." (Abstract) ASGSB Bull., 11, 72 (1997).

Relationship of Morphogenesis and Mineralization to Gravitaxis in Spaceflown Algae

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No Co-Is Assigned to this Task

Funding:

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Initial Funding Date: not available	Expiration: not available
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$10,000	

Flight Information:

Experiment ID: 9601207
 Flight Assignment: ISS/TBD
 Responsible NASA Center: Ames Research Center

Task Description:

Pleurochrysis carterae is a marine alga covered with calcified interlocking scales called coccoliths, which are species-specific and produced in Golgi cisternae in a precise and orderly manner prior to positioning outside the cell membrane. This calcifying unicellular green alga provides an excellent system for the study of biologically directed calcification without the interference of systemic effects.

P. carterae is also negatively gravitaxic, a phenomenon caused by a gravity-induced hydrodynamic torque dependent upon the shape and density distribution of the organism. Alterations under altered gravities of morphogenesis and scale production and/or mineralization should be reflected in altered gravitaxis. We propose to use *P. carterae* to examine the effect of microgravity on biomineralization, and the relationship of biomineralization and morphology to motility and gravitaxis. These studies will include effects of microgravity on: cell reproduction and development (including aspects of intracellular polarity, and persistence of changes in future generations); coccolith production, morphogenesis, and its polysaccharide-mediated mineralization; and the swimming behavior (direction and speed) of this unicellular photosynthetic organism. Techniques will include: flow cytometry, transmission and scanning electron microscopy, computer assisted planimetry and 3D reconstruction, ultrastructural immunogold localization, X-ray diffraction, microprobe, motion analysis of videotapes, and gyrotaxic focusing.

Investigation of these coccolithophorids will yield results that are significant for materials science, ecology, and evolution, as well as for gravitational and space biology. Most directly relevant to NASA's Space Biology Program, this research asks whether loading and unloading cells by altering the force of gravity has a direct influence on growth rate, development, and mineralization of microorganisms. If positive, the results will open an entire new field of micro-modeling, analogous to currently employed bacterial and zooplankton assays of carcinogenicity and toxicity.

Although no funding was received for 1997, we completed the Phase A activities of the project as defined in May/June 1997 by Ames Research Center. In these activities, the maximum-minimum time periods of culture, and the maximum/minimum cell concentrations, and so on were defined. Such definition was necessary for ARC to make hardware/flight decisions for this experiment.

The coccolithophorids are extremely important in the cycling of carbon in the biosphere. As photosynthetic organisms, coccolithophorids remove CO₂ from the environment during the photosynthetic process; during mineralization, CaCO₃ is formed from bicarbonate and CO₂ is released. Although we have not investigated this possibility, these algae may be of some use in removing CO₂ from the shuttle/station environment. Another area of significance to the Bioregenerative Life Support program is the possible nutritive use of *P. carterae*. Photosynthetic algae are sources of nutrients and vitamins, and *P. carterae* might even provide a calcium supplement in space.

This project is also of significance to the Biotechnology portion of the Microgravity program in terms of (1) biologically directed mineralization, and (2) assembly of nanostructures.

This study is the first in a new field of micro-modeling in space, analogous to currently employed bacterial and zooplankton assays of carcinogenicity and toxicity on Earth. In our proposed study, we plan to use *P. carterae* as a model for studying the relationship between gravity and growth and form, secretion, cell polarity, calcium metabolism and extracellular matrices, cell growth, and reproduction.

FY97 Publications, Presentations, and Other Accomplishments:

Duke, P.J. and Montufar-Solis, D. "The relationship of morphogenesis and mineralization to gravitaxis in spaceflight algae." Seminar presented at Ames Research Center (May 28, 1997).

Intermittent Administration of Parathyroid Hormone: Countermeasure for Loss of Bone Mass and Strength During Spaceflight

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Funding:

UPN/Project Identification: 106-50-01
Initial Funding Date: not available
Students Funded Under Research: 0
FY 1997 Funding: \$10,000

Solicitation: 96-OMSA-01
Expiration: not available
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9601227
Flight Assignment: TBD
Responsible NASA Center: Ames Research Center

Task Description:

Loss of weight bearing during space flight results in osteopenia. Decrements in bone mineral of 3-10% after 75-184 days in space have been reported. The loss of bone associated with space flight or skeletal unloading occurs as a consequence, in part, of decreased bone formation. During flight, tibial periosteal bone formation and humeral cancellous bone formation decrease by 41% and 38%, respectively. This leads to a deficit in bone mineral and a decrease in bone strength, thus increasing the risk of fracture. Attempts to prevent the bone loss associated with space flight have met with limited success. Intermittent administration of parathyroid hormone can stimulate bone formation, increase bone mass, and improve bone strength. Using a ground-based model simulating the weightlessness of space flight, we have shown that intermittent administration of PTH can prevent the loss of cancellous bone, reduce the decrement in cortical bone formation and prevent the normal deficit in femoral mass induced by skeletal unloading. The objective of this proposal is to determine whether intermittent administration of PTH can also prevent the inhibition of bone formation, deficit in bone mass and loss of bone strength that occur during space flight. Animals will be implanted with Alzet osmotic minipumps attached to small bore tubing sequentially loaded with boluses of an aqueous solution of PTH separated by oil in order to effect intermittent administration of hormone during spaceflight. Bone structure, formation rates, mass and strength will be compared in flight and ground-based control animals treated with either PTH or vehicle.

This project is in its early stages.

Crew Member and Crew-Ground Interactions During International Space Station Missions

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Funding:

UPN/Project Identification: 106-20-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$25,000

Flight Information:

Experiment ID: 9601096

Flight Assignment: ISS/TBD

Responsible NASA Center: Johnson Space Center

Task Description:

During International Space Station (ISS) missions, international crews will be engaged in complicated activities over long periods of time. A number of interpersonal issues likely to impact on these missions must be addressed in order to ensure healthy crew member interactions and optimal performance. A review of the literature of space analog studies on Earth, anecdotal reports from previous space missions, and the principal investigator's own work involving astronauts and cosmonauts have isolated crew tension, cohesion, and leadership as important interpersonal issues. Cultural and language differences among crew members and the relationship of space crews with monitoring personnel in mission control also are important factors that influence crew performance and well-being.

Hypotheses related to these interpersonal issues will be tested by having the crew members and personnel in ground control answer a series of questions from three standard mood and interpersonal group climate questionnaires, a critical incident log, and a culture and language questionnaire. The culture and language questionnaire will be administered once before each mission. The other measures will be completed on a weekly basis before, during, and after each mission and will take 15-20 minutes to fill out. By using an interrupted time-series analysis and a number of predicted correlations, a test of the hypotheses will be made and discussed.

During the definition phase, contact was made with key support staff and project scientists at NASA/JSC. In addition, a timeline and budget were submitted for final approval. Finally, planning for and the initial development of a web-based computerized questionnaire for use in space as well as on the ground were begun.

In planning for future manned space missions involving international crews of men and women, it is important to prepare for the occurrence of interpersonal issues that might negatively affect the relationships of crew members and their ability to carry out mission goals. These factors include interpersonal tension, crew

cohesion, leadership roles, culture and language differences, and the relationship between space crews and monitoring personnel on the ground. These factors constitute the variables of interest in this study. We believe our findings will be of use in developing better selection, training, and in-flight support procedures during future space missions as well as helping space travelers readjust better to their families and to society after they return to Earth.

The interpersonal interactions of long-duration, multi-national space crews constitute a laboratory of small group behavior that tells us a great deal about ways in which groups of people on Earth can relate with a minimum of tension and improved cohesion when they are under stress. In addition, the ability of people from previously opposing political blocks to engage in complex activities, such as undertaking a space mission, serves as a model for international cooperation on Earth. Thus, this research project will teach us a great deal about ourselves and our ability to relate with one another despite cultural and political barriers.

FY97 Publications, Presentations, and Other Accomplishments:

The following are related publications and presentations that were developed during Phase 1B and may be of interest:

Kanas, N., Weiss, D.S., Marmar, C.M., Crewmember interactions during a Mir space station simulation. *Aviation, Space, and Environmental Medicine*, 1996; 67:969-975.

Kanas, N., Psychosocial value of space simulation for extended spaceflight. *Advances in Space Biology and Medicine*, 1997; 6:81-89.

Kanas, N., Psychosocial findings from recent space simulation studies. *Aviation, Space, and Environmental Medicine*, 1997; 68(7):640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 157).

Kanas, N., Weiss, D.W., Marmar, C.M., Grund, E.M., Applicability of simulation studies to psychosocial issues in space. *Aviation, Space, and Environmental Medicine*, 1997; 68(7):640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 158).

Gushin, V.I., Zaprisa, N.S., Kolinitchenko, T.B., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., Kanas, N., Content analysis of the crew communication with external communicants under prolonged isolation. *Aviation, Space, and Environmental Medicine*, 1997; 68(7):640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 159).

Gushin, V.I., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., Kanas, N., Subject's perceptions of the crew interaction dynamics under prolonged isolation. *Aviation, Space, and Environmental Medicine*, 1997; 68(7):640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 160).

Kanas, N., Psychosocial support for Astronauts. *Aviation, Space, and Environmental Medicine*, 1997; 68(10):964. (From "1997 Abstracts of AsMA Scientific Sessions: Discussion Panel Abstracts", 1997, Abstract No. 554).

Kanas, N., Psychosocial issues affecting Astronauts and Cosmonauts in space. *Aviation, Space, and Environmental Medicine*, 1997; 68(10):964. (From "1997 Abstracts of AsMA Scientific Sessions: Discussion Panel Abstracts", 1997, Abstract No. 555).

Kanas, N., Psychosocial issues affecting crews during long-duration international space missions. Paper presented at the 12th Man in Space symposium, 1997; Washington, D.C.

Kanas, N., Crewmember and crew-ground interactions. Paper presented at the Phase 1 Research Program Interim Results Symposium, 1997; Johnson Space Center, Houston, TX.

Space Flight Effects on Fungal Growth, Metabolism and Sensitivity to Antifungal Drugs

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Funding:

UPN/Project Identification: 106-50-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 15,000

Flight Information:

Experiment ID: 9601240

Flight Assignment: TBD

Responsible NASA Center: Johnson Space Center

Task Description:

This project supports NASA's Life Sciences Division's major goals of using microgravity during space flight to understand its influence on fundamental biologic processes in eukaryotic yeast cells, and its influence on sensitivity of fungi to antifungal drugs, which will contribute to a safer, productive human presence in space. The Environmental Health Program's goals are supported by developing a eukaryotic yeast model for determining the effect of microgravity upon fungal sensitivity to antifungal drugs, which impacts crew health. The Biology Program's goals are supported by using the eukaryotic yeast model to study the effect of microgravity upon the cytoskeleton (cell wall) and cellular functions. Data gathered will improve our knowledge of eukaryotic cellular functions and health on Earth.

Our hypothesis is that microgravity will cause subtle to profound affects on fundamental eukaryotic cellular and metabolic processes. Using eukaryotic yeast cells as a model, we propose to study the effect of microgravity during space flight on metabolic functions necessary for yeast identification and the action of antifungal drugs. Identification and sensitivity to antifungal drugs in microgravity is important to crew health and mission success. Other influences of microgravity to be studied include its effect upon chitin deposition, ultrastructure of the cell wall and organelles, physiologic pathways, morphologic and reproductive expression, and antifungal drugs as molecular antagonists.

Multiple strains of *Saccharomyces cerevisiae*, *Candida albicans*, *Rhodotorula rubra*, and other yeasts will be studied using Vitek (bioMerieux Vitek, Inc.) modified yeast biochemical cards. During shuttle flight, carbohydrate and nitrate assimilation and sensitivity to antifungal drugs will be determined. The test system is well contained, weight and volume requirements are low, and crew involvement is minimal.

This project was in definition stage during FY97. Preliminary paperwork was submitted for final approval. Official start date should be in March 1998.

This research will offer an understanding of the effect of microgravity on the basic biological processes of fungi especially in response to anti-fungal drugs. This understanding will enhance our management of fungal infections in immunologically compromised individuals in space and on earth.

Human Orientation and Sensory-Motor Coordination in Prolonged Weightlessness

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Funding:

UPN/Project Identification: 106-20-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$30,000

Flight Information:

Flight Assignment: ISS/UF-2

Responsible NASA Center: Johnson Space Center

Task Description:

This International Space Station Experiment (Utilization Flight Experiment 085) merges and extends two investigations originally developed for the 1998 Neurolab space shuttle mission. The science team includes experienced investigators from the USA, Canada, France, and Italy. On earth, gravity provides a reference that influences how we recognize objects in our field of view and perceive their shape and orientation. It also influences our expectations of how objects will behave when thrown or dropped. The orientation of familiar visual objects and surfaces in turn influence our own perceived orientation. Gravity also helps the central nervous system align the various frames of reference used in movement control. All of these processes are fundamentally altered in weightlessness, as evidenced by the visual reorientation, inversion, and proprioceptive illusions frequently reported in-orbit by astronauts, and also by the surprisingly long lasting aftereffects seen, particularly after prolonged space flight. This experiment utilizes the Human Research Facility Computer Workstation and virtual environment generation accessories first developed for the Neurolab as a tool to study these processes during and after long duration (3 month) orbital flight. Restrained and free-floating subjects wear a wide field of view, color stereo head mounted display, and view controlled visual scenes in five different test paradigms which assess: (1) the influence of scene symmetry, rotation, haptic cues, and expected orientation on static and dynamic self tilt (Virtual Tilting and Tumbling Room Tests); (2) the onset of x-axis illusory linear self-motion without haptic cues (Linear Vection Test); (3) the effect of perceived orientation on visual object recognition and shape recognition (Object Recognition Tests); (4) whether information used in grasping

remembered objects is stored in head fixed, body fixed, or exocentric reference frames (Virtual Grasping Test); and (5) how the timing of catching movements depends on anticipation of downward acceleration (Virtual Catching Test). The 12-30 minute long test modules are based on existing 1-G paradigms, require little set-up time, and can be selected and performed by an astronaut in an automated fashion using Session Manager software. Two preflight, three inflight, and three postflight performances of each test are planned on each ISS increment.

Experiment Definition Phase is just getting underway (1Q, 1998). Investigators have met in Boston and Houston to review experiment requirements, the performance of the suite of VR workstation accessories constructed for Neurolab, techniques which could be used for preflight and onboard crew refresher training exploiting the capabilities of the multimedia workstation, and to discuss the feasibility of remote science support activities in Boston and Paris during the mission. An experiment document is in preparation.

The vertebrate nervous system evolved in an environment where the stimulus to the body's multiple gravireceptive senses invariably changed whenever the orientation of the body was altered. Experiments conducted in orbital flight allow us to remove static gravireceptor cues to the orientation of the head, body, and limbs, so we can better understand the role of gravity in fundamental sensory, motor, and cognitive processes subserving spatial orientation and movement control in daily life on Earth. We usually only become aware of these functions when they are compromised by inner ear or central nervous system disease. If this happens, our everyday lives are profoundly affected. More than 90 million Americans suffer from some type of balance disorder. Patients often have difficulty walking at night, cannot see clearly (particularly when moving), cannot safely drive, and suffer injurious falls and sometimes incapacitating bouts of vertigo and nausea. Humans with hippocampal lesions or Alzheimer's disease show impairments on a wide variety of spatial and navigational tasks. There is currently considerable interest in the development of new methods for evaluating a patient's ability to use visual and proprioceptive cues to maintain balance and orientation, and for improving motor control function via rehabilitative training. Portable head mounted displays, akin to those used in this experiment, may well prove useful for such testing and training.

Automated Use of DNA Probes for Rapid Detection of Bacteria in Water

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No Co-Is Assigned to this Task

Funding:

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Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$30,000

Flight Information:

Experiment ID: 9601212

Flight Assignment: ISS/TBD

Responsible NASA Center: Johnson Space Center

Task Description:

We plan to develop an automated DNA probe technology for the monitoring of bacteria in water for use during space flight. This simple water quality assay will provide a rapid, specific, and sensitive means of identifying and measuring the number of total bacteria, total coliform bacteria, *Escherichia coli*, *Burkholderia (Pseudomonas) cepacia*, *Methylobacterium spp*, and other heterotrophic bacteria in water samples. Using this approach, a completed test should be attainable in less than 8 hours, with a sensitivity of 100 CFU/100 ml of water or better. Three objectives will be addressed by this proposal. The first will be to define a common set of protocols for use of DNA probes against specific target bacteria and bacterial groups. The second objective is to analyze the application of chemiluminescent compounds as reporters of hybridization and automate the hybridization protocol. The third objective is to evaluate the automated system in both ground-based and microgravity environments. Potential applications of this research include tests for bacterial contamination of potable, recreational, sanitary, and industrial water. This research will produce a near real-time monitoring test for field, home, and industrial use that will be easy-to-use, rapid, selective, and sensitive. Furthermore, the automation of this assay will allow for a microgravity operation that will not greatly impact crew time or spacecraft resources. This rapid technology can be directly applied to the current space shuttle program as a cost saving measure when lengthy preflight bacterial analysis would result in a launch delay. Development of this technology will be a benefit for NASA, private, and public sectors, consistent with a major directive of NASA's Strategic Plan for the development and transfer of technology to the private sector.

This is a new start for FY97 with funding available for the last five months of the reporting period. Initial efforts includes formation of the research team. Dr. George Fox from the University of Houston joined the team and will assist in the design of the probes.

Phase I activities include requirement definition, probe development, assay optimization, and evaluation of the probes. During the last five months of FY97, we identified *E. coli*, *Burkholderia cepacia*, *Methylobacterium*

spp, total fecal coliforms, and total heterotrophs as the microbes and microbial classes for probe development. DNA from *B. cepacia* was isolated and some initial sequencing was completed in preparation for probe design. No technical problems have arisen, and FY 98 should see significant progress in probe development for the targeted microbes.

The adverse effects of microorganisms on the health, safety, and performance of people on Earth and in space is well documented. Since 1973, more than 30 new infectious diseases have been identified, and numerous known ones have re-emerged. CDC has initiated a new peer-reviewed journal *Emerging Infectious Diseases* in response to increasing importance of infectious disease world-wide. Even in the US we have 9,000 deaths a year from cases of food poisoning and 900 deaths per year from microbial contamination of water.

The need for rapid, reliable tests for microbial agents has never been more important than today. Technology for rapid diagnostics and environmental analysis has not been available. Most technologies commonly used as the "gold standards" in both clinical and environmental laboratories are based on culture techniques. This technology has been used for more than 100 years and is not rapid enough for today's needs. Much work is being conducted to help alleviate this urgent problem. In recent years, considerable progress has been made in molecular approaches to addressing this problem. A DNA probe is one such technology that can provide relatively rapid analysis of microbes and is not based on culture technology. The successful completion of this NASA research task should provide probe technology for the rapid detection and identification of waterborne bacteria. This could be used in the food and beverage industry, pharmaceuticals, semiconductor industry, water producing companies, and others. The technology can also be used on clinical applications, such as urinary tract infections, allowing for a more rapid treatment and better clinical outcome.

Incidence of Latent Virus Shedding During Space Flight

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-20-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 150,000

Flight Information:

Experiment ID: 9601409

Flight Assignment: ISS/TBD

Responsible NASA Center: Johnson Space Center

Task Description:

Latent virus reactivation may be an important threat to crew health during extended space missions as crewmembers live and work in a closed environment. Infectious disease risks associated with latent viruses are not mitigated by a quarantine period before space flight or by prophylactic treatment, as are most bacterial, fungal, viral, and parasitic agents. Herpesviruses are the most readily recognized of the latent viruses and are the leading infectious cause of blindness in the United States. The establishment of latency and the subsequent reactivation are not well understood, but decrements in the immune system are known to increase the incidence and duration of viral reactivation and shedding of some latent viruses. An increasing body of evidence indicates that the physical and psychological stressors associated with space flight cause anomalies in the human immune response. The hypothesis to be tested is that space flight will increase the incidence and duration of herpesvirus reactivation and shedding in saliva. The investigation will require collection of saliva and urine samples from mission participants before, during (saliva only), and after space flight. The saliva and urine samples will be analyzed using the polymerase chain reaction (PCR) to detect the presence of four important herpesviruses (herpes simplex type-1 and type-2, cytomegalovirus and Epstein-Barr virus). The PCR technique allows both symptomatic and asymptomatic shedding to be detected. Serum levels of antibody to the four herpesviruses under study will be determined by enzyme-linked immunosorbent assay (ELISA) before and after flight. The specific goals of the study are to (1) collect saliva, urine, and serum specimens from crewmembers at appropriate intervals before, during (saliva only), and after space flight; (2) analyze for herpesvirus DNA in saliva and urine and determine serum antibody titers; (3) quantify shedding frequency and duration during flight and compare them with ground-based control values; (4) determine if shedding patterns of individual herpesviruses during flight differ from ground-based values; and (5) develop ground-based models. Data from this study will support and expand information on latent virus reactivation during space flight. Information gained from experiments performed on Space Shuttle missions will be essential for development of pharmacological countermeasures for long duration missions. This data will be essential for the overall infectious disease risk assessment associated with space flight.

The objective of the viral reactivation project is to determine the effects of space flight upon viral reactivation and shedding patterns. Initially, this investigation was conducted on US crewmembers during Space Shuttle flights. This allowed us to study the phenomenon on short duration missions. Results are given below:

Space Shuttle: Saliva and urine samples were collected from four Space Shuttle crews (STS 82, 83, 85, and 94) in 1996-97. Reactivation and shedding of latent herpesviruses were assessed in these samples by extracting DNA, amplifying with specific primers using PCR, and finally detecting viral DNA using Digene Sharp Signal Detection System. Epstein-Barr virus (EBV) was selected as the target virus to study initially in saliva. Shedding of EBV was observed in Shuttle crew members during flights; frequency was highly variable and similar to shedding observed in ground controls. Urine samples were collected before and after five Space Shuttle missions (STS 75, 76, 77, 78, and 79) and processed for DNA extraction and detection of Cytomegalovirus. The results are being analyzed.

Since access to space is very limited, ground-based analogs were used.

Lunar/Mars Life Support Test Project (LMLSTP) Phase IIA: Saliva samples and urine samples were also collected from four crewmembers who participated in a 60-day chamber study. Saliva samples were analyzed for EBV using specific primers. Viral reactivation was found to be more frequent in the earlier period of isolation. Of the four crewmembers, EBV DNA was detected quite frequently in one crewmember throughout the study, only in the initial part of the study in the second crewmember, rarely in the third, and not at all in the fourth crewmember.

LMLSTP Phase III: A 90-day chamber study with four crew members participating in the viral reactivation study will be completed by late December 1997. Saliva, blood, and urine samples are being collected for this study.

Antarctic 1994: Reactivation of latent viruses may be an important health threat to isolated teams in extreme environments such as Antarctic expeditioners or space crews. Compromise in immune function under such conditions could increase the incidence and duration of viral reactivation and shedding. We studied 15 subjects (14 men and one woman) at the Australian Antarctic outposts Davis and Mawson to assess the reactivation and shedding of herpesviruses in saliva during an 8-month winter-over period. Each participant provided saliva samples before, during and after the winter-over period as follows: Each subject was asked to collect the samples first thing in the morning before eating or drinking anything. Samples were collected approximately once a week before and after the winter-over and three times a week during the study. A total of 642 saliva specimens were collected, 233 from 5 subjects at Mawson and 409 from 10 subjects at Davis. These specimens were stored at -70°C until the end of the winter-over period. They were shipped on dry ice to NASA-Johnson Space Center in Houston for analysis. Each sample was concentrated to 200 μ l with a 100k Microsep concentrator. Epstein Barr virus DNA was extracted from each sample with Qiagen's QIAamp 96 Spin Blood Kit, amplified by PCR and detected with Digene Sharp Signal detection system. EBV DNA was detected in a total of 86 (15.03%) saliva samples, of which 45 (21.1%) were from Mawson and 41 (11.4%) from Davis. All the subjects included in the present study shed EBV at least once in their saliva samples. Although the temporal shedding pattern varied in these subjects, the EBV DNA was detected more frequently in the saliva samples collected during the first half of the winter-over than during the latter half. In four subjects (two each from Mawson and Davis), viral DNA was shed in 28% to 38% of the samples collected from each subject. However, two subjects from the Davis outpost shed only once during the winter-over period. Reactivation of viral shedding was correlated with immune status of these subjects by studying cell-mediated immune (CMI) response. Cutaneous CMI multitest were performed on the forearm of each subject quarterly over the 8-month winter-over period. These results showed considerable variability in subject responsiveness to specific antigens at different times. However, CMI response generally was lower during the study. Based upon these results of cutaneous test, 3 subjects reacted with 0 or 1 antigens and were considered in anergy, and 7 reacted with 3 or less antigens and were considered in a hypoery category. We conclude that extreme cold isolation and other stress factors may cause an increase in EBV reactivation early during winter-over periods, and that this finding may be associated with lower CMI response.

Gastrointestinal Function During Extended Duration In Space

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Funding:

UPN/Project Identification: 106-20-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$10,000

Flight Information:

Experiment ID: 9601110

Flight Assignment: ISS/TBD

Responsible NASA Center: Johnson Space Center

Task Description:

The combined effects of fluid shifts, fluid loss, decreased fluid consumption, and postural changes in microgravity are postulated to decrease splanchnic blood flow, decrease gastrointestinal (GI) motility, and thereby decrease absorption of essential nutrients and therapeutic countermeasures across the intestinal wall. Decreases in gastric emptying rate are suspected of causing variability in the absorption of oral acetaminophen during short duration flights of up to 5 days (Cintrón et al., 1987). The contribution of GI function to negative energy balance will adversely affect crew health and confound the effectiveness countermeasures (e.g. exercise and fluid loading, pharmacologic agents) during long-duration missions as well as landing and egress operations. We hypothesize that GI function in microgravity will be altered as a result of decreased GI motility. Therefore, the overall objective of this ongoing inflight investigation is to determine, using two well characterized noninvasive probe compounds, the effect of space flight on GI motility, as measured by changes in the rates of gastric emptying and intestinal transit time. This information will be useful for designing and delivering safe and effective pharmacologic treatment during space flight.

Gastrointestinal function will be determined using a lactulose breath hydrogen test, a noninvasive and indirect measure of gastrointestinal motility. GI motility will be evaluated by recording a rise of hydrogen concentration in the breath after consumption of lactulose, a nondigestible sugar. In addition, the protocol will also evaluate pharmacokinetic changes during space flight using oral acetaminophen as a representative drug. The rate of gastric emptying will be evaluated by following the appearance of acetaminophen and its metabolites in saliva and urine after ingestion. The absorption and bioavailability of oral medications like acetaminophen are expected to be reduced during flight.

In response to reviewers' comments about the possibility of bacterial overgrowth during flight, a glucose hydrogen breath test to detect small bowel bacterial overgrowth and a ¹³C-Urea Breath to determine the frequency

of active *Helicobacter pylori* gastric colonization have been introduced in the experimental design to be completed by the crew before and after flight.

The in-flight data collected on Mir 18 (2 crewmembers) indicate a trend in GI disturbances. An increase by at least 100% in the gastrointestinal transit time (GITT) (determined from breath hydrogen) during flight was observed in both crewmembers, indicating a decrease in GI motility. Both crewmembers, who repeated the test three times in-flight, exhibited a sustained increase in GITT throughout the duration of the mission. This is in agreement with an earlier observation of a 63% increase in the GITT during antiorthostatic bed rest.

Breath methane and hydrogen levels were several fold higher during flight than on the ground. Thirty to fifty percent of the general population excrete methane, possessing methanogenic bacteria in their distal colons that convert hydrogen to methane. Endogenous protein or glycoprotein changes may also contribute to methane response. In this study, one crewmember excreted low levels of methane (<40 ppm) during pre- and postflight testing. The second crewmember did not excrete methane levels >3 ppm either pre- or postflight. Interestingly, both the Mir 18 crewmembers excreted high levels of methane during all three in-flight collections: baseline levels during flight ranged from 1784 ppm to 6661 ppm, compared to 0-26 ppm on the ground. Methane production indicates gut stasis, change in gut-wall bacterial flora, or a combination of both. It is noteworthy that the toxicology lab recorded high methane levels in the Mir 18 air samples as well. The source of methane, although unknown at this time, may be the crew.

High breath methane and hydrogen levels are also associated with bacterial overgrowth in the GI tract and a possible proliferation of pathogenic bacteria, *Helicobacter pylori* (Minocha, et al., 1994). It was reported that the crewmembers received prophylactic treatment with bifidobacterium for disbacteriosis 60 days prior to flight. Their fecal bacterial flora immediately before flight were normal indicating that the disbacteriosis was effectively treated with bifidobacterium. No anomalies in the bacterial flora were noticed after return from flight (personal communication by Dr. Lizco, IBMP, Moscow). These results indicate that future studies should focus on examining some of these variables of the GI physiology during Mir station flights.

GI function is key to absorption and availability of nutrients and oral medications. Absorption and bioavailability of acetaminophen was variable during the Mir 18 flight and correlated well with GI motility. This supports earlier findings from DSO 622 (GI Function During Extended Duration Space Flight) and DSO 458 (Salivary Acetaminophen Pharmacokinetics).

Results from these studies are very important for furthering the understanding of GI function in microgravity and for developing countermeasures. A proposal has therefore been submitted to that effect and was approved for funding. The overall objective of the proposed research is to identify specific physiological and pharmacokinetic changes in crewmembers during long missions (e.g., ISS). This information will be useful in designing safe and effective pharmacologic treatments for space flight.

The experiment document for this proposed work has been initiated in collaboration with Lockheed Martin scientists.

The results of this study would help in understanding the mechanisms which affect the GI physiology. Benefits would include the development of appropriate treatments for eliminating the GI disturbances noticed both on Earth and in space. In addition, the proposed research employs non-invasive techniques for understanding the underlying causes of GI disturbances which could potentially have great benefits on Earth. Furthermore, development of novel pharmacological dosage forms for countermeasures in the treatment of GI disturbances in space could also be very useful on Earth.

Development of Gravity Sensitive Plant Cells (Ceratodon) in Microgravity

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-20-01
Initial Funding Date: not available
Students Funded Under Research: 1
FY 1997 Funding: \$20,000

Solicitation: 96-OLMSA-01
Expiration: not available
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9601445
Flight Assignment: ISS/TBD
Responsible NASA Center: Ames Research Center

Task Description:

Protonemata of the moss *Ceratodon* are tip-growing cells that grow up in the dark. This cell type is unique compared to cells in almost any other organism, since the growth of the plant cell itself is completely oriented by gravity. Thus, both the processes of gravity sensing and the gravity response occur in the same cell. Gravity sensing appears to rely upon amyloplasts (starch-filled plastids) that sediment. This sedimentation occurs in specific zones and plastid zonation is complex with respect to plastid morphology, distribution and gravity. Microtubules restrict the extent of plastid sedimentation (i.e., they are load-bearing). Since gravity plays crucial roles in the growth and organization of this highly specialized cell, it is relevant to determine whether this cell differentiates normally in microgravity.

Light also is important since (1) apical cells have a phytochrome-based positive phototropism, (2) light quality influences plastid zonation and sedimentation (photomorphogenesis), and (3) red light suppresses gravitropism at higher but not lower light intensities.

Protonemata are readily cultured in agar in sealed containers in the dark enabling large sample sizes and reasonable replicates. They can be grown in a passive container and fixed in position in space. Thus, this system is rich both in biological questions and in practical advantages for microgravity studies.

This proposal describes flight experiments that build upon data and questions arising from a previous space flight experiment (STS-87). Specific objectives include a determination of (1) whether both wild-type and the wrong way response mutant (that grows down instead of up) are equally randomly oriented in cultures grown in the dark in microgravity; (2) whether plastid zonation and morphology and cell growth and differentiation in both the light and the dark are comparable in microgravity and ground controls; and (3) whether phototropism has a higher fidelity in space and whether gravitropism can be separated from phototropism at low light intensities in microgravity.

During FY 1996-1997, funds were provided to facilitate the further definition of the protocols and questions in preparation for the space flight experiment. A major objective of the flight experiment is to characterize the distribution of amyloplasts in response to the vector or the absence of 1-G. Analysis of upright and inverted stationary cells on Earth shows that amyloplasts fall along the length of the cell but that not all do so. Since we have shown that microtubules are load bearing for these plastids, our goal is to compare the distributions of plastids in microgravity and on a clinostat with upright and inverted controls. If amyloplasts are under tension, then amyloplasts should be located closer to the tip of the cell in microgravity, whereas if tension is not a factor at 1-G, then amyloplasts should be randomly distributed in microgravity. We will also test whether clinostat rotation (on Earth) mimicks microgravity with respect to plastid distribution. Efforts towards defining this analysis have included optimization of protocols for visualizing and quantifying plastid distribution.

A second objective is to examine the interactions between phototropism and gravitropism. Purpose-built hardware enabling illumination and fixation *in situ* will fly on STS-87. Further definition of the subsequent experiment involves characterization of the gravity response in low intensity red light and white light of a range of intensities.

This research primarily addresses fundamental questions in gravitational biology. One thrust is to shed light on how gravity controls the organization of cells. The flight experiment will help in understanding how the components of cells are regulated so as to not be simply stratified with respect to gravity based on their densities.

Another thrust is to understand how cell growth which is localized to one pole of a cell is regulated by the environment, i.e., how tip growth is modulated. Plant productivity depends in a major way on tip-growth for root growth and for fertilization and fruit and seed production. Knowledge about the regulation of tip-growth would thus be helpful for understanding one of the bases of agronomic yield.

FY97 Publications, Presentations, and Other Accomplishments:

Kern, V.K. and Sack, F.D. "Relationship between gravitropism and phototropism in protonemata of the moss *Ceratodon*." (Abstract) *Plant Physiol.*, 114: (Suppl) Abstract 623 (1997).

Renal Stone Risk During Space Flight: Assessment and Countermeasure Validation

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Funding:

UPN/Project Identification: 106-20-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$80,000

Flight Information:

Experiment ID: 9601057

Flight Assignment: ISS/TBD

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: UCD, BCR, In-flight Urine Storage Tube Kit, Dried Urine Chemistry Hardware

Task Description:

Human exposure to microgravity results in a number of physiologic changes. Among these changes are the alterations in renal function, fluid redistribution, bone loss and muscle atrophy, all of which contribute to an altered urinary chemical environment. In-flight changes previously observed include decreased urine volume or increases in urinary calcium, phosphate, potassium, and sodium excretion which could all potentially exaggerate the risk of renal stone formation. The formation of a renal stone in-flight could have severe consequences for both the health of the crewmember and the mission success. This study involves pre-, in-, and post-flight 24-hr urine collections to assess the renal stone-forming potential that exists during space flight and to determine how long after flight increased risk exists. In addition to the analyses of mineral components, we will also determine levels of the naturally occurring urinary protein inhibitors of calcium crystallization. Using these quantitative measures of risk will allow us to quantitatively test the effectiveness of potassium citrate and/or hydration as countermeasures to the altered urinary chemical environment exhibited during space flight. This study addresses NASA's objectives to optimize crew safety, well-being, and performance by understanding the effects of prolonged hypercalciuria and the development of prophylactic methods to minimize/eliminate the risk of renal stone formation during space flight.

This investigation directly builds on our previous work describing the increased risk of renal stone development during and immediately after space flight. The complexity, expense, and visibility of the human space flight program necessitates that every effort is made to assure the success of each mission. During the last year, one astronaut developed a renal stone at approximately two weeks following a Space Shuttle flight. Based on our data from earlier studies all crewmembers and flight surgeons have been advised as to the beneficial effect of increasing fluid intake to increase urine volume in order to minimize the risk of an in-/postflight occurrence of

renal stones. Particular attention has been given to those crewmembers participating in long-duration missions aboard the Mir space station.

Lessons learned from these early investigations have led us in the last year to modify the current flight hardware to maximize the crewmember's time and the quality of the science returned from space flights. Currently in progress are analyses of the renal stone dietary and urinary data from the long-duration missions, planning for the in-flight testing of pharmacologic countermeasures to minimize the risk of renal stone development, investigations into the effects of space flight on the naturally occurring urinary protein inhibitors to renal stone formation and the first in-flight testing of the dried urine chemistry hardware investigation.

Renal stone disease affects approximately 12% of the human population on Earth with recurrence rates of renal stones reaching 75% in those individuals. Morbidity is high and related health costs have been estimated to exceed 2 billion dollars a year. Understanding how the disease may form in otherwise healthy crewmembers under varying environmental conditions may lead to additional clues as to how crystals form in the urine and develop into renal stones. The development of the Dried Urine Technology will enhance the capability reliability and quality of Life Sciences flight hardware, enable new types of scientific investigations in space not presently possible, minimize costs, schedule and program risk to life sciences flight experiments, as well as ensure the promotion of technology transfer to the commercial sector. This enhanced technology not only eliminates the requirement for a refrigerator/freezer and a centrifuge on board the spacecraft but opens the possibility of new real-time diagnostic techniques that have numerous Earth-based and flight applications. This new technology provides a breakthrough to the current limitations of available technology as well as providing benefits for the private and public sectors.

FY97 Publications, Presentations, and Other Accomplishments:

Chen, Y.M., Pietrzyk, R.A., and Whitson, P.A. "Quantification of urinary uric acid in the presence of thymol and thimerosal by high-performance liquid chromatography." *J. Chromatography*, vol. 763, 187-192 (1997).

Lane, H.W., Whitson, P.A., Putcha, L., Baker, E., Smith, S.M., Gretebeck, R.J., Schoeller, D.A., Davis-Street, J., Pak, C.Y.C., Pietrzyk, R.A., Nimmagudda, R.R., DeKerlegand, D.E., Stewart, K., and Bourne, D.W.A. "Extended duration orbiter medical project final report. Regulatory physiology." NASA Publication (In Press).

Whitson, P.A. "Renal stone risk assessment in astronauts." Phase I research results symposium, Gilruth Center, NASA/Johnson Space Center, Houston, TX (August 5 - 7, 1997).

Whitson, P.A., Pak, C.Y.C., and Pietrzyk, R.A. "Renal stone risk during space flight." 12th Man in Space Symposium. The Future of Humans in Space. Washington, DC (June 8 - 13, 1997).

Whitson, P.A., Pietrzyk, R.A., and Pak, C.Y.C. "Renal stone risk assessment during space shuttle flights." *J. Urology*, 2305-2310 (1997).

Direct Measurement of the Initial Bone Response to Spaceflight in Humans

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Funding:

UPN/Project Identification: 106-30 (E074)
Initial Funding Date: 1992
Students Funded Under Research: 0
FY 1997 Funding: \$270,000

Solicitation: AO-OSSA-84
Expiration: 1997
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 284074
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center

Task Description:

The skeleton is constantly being broken down and rebuilt, with the processes normally occurring at equal rates. Space flight upsets this equilibrium, and the resulting imbalance between breakdown and reformation could cause lasting changes in the amount of bone, even after a short mission. The net cumulative effect of multiple short-term flights may, in fact, be similar to that of extended exposure, creating concern for the health of astronauts who fly multiple short missions or who will be involved in the assembly phase of the International Space Station. This experiment is designed to interpret long-term effects of microgravity, based on each astronaut's individual inflight response to the short-term exposure to space.

The data collection phase of the experiment was done from March - October 1996. This included preflight data collection from L-90 to launch, 17 days inflight, and postflight data collection to about R+60. Experiment protocols included the collection of multiple serum, urine, and stool specimens during these periods as well as dietary intake information. Approximately 25 blood samples and hundreds of urine specimens were obtained for each crewmember participating (two mission specialists and two payload specialists, all male), making it the most complete metabolic balance studies in space flight since the Skylab missions. The crew also took pills containing a stable (non-radioactive) isotope of calcium from 10 days preflight to 7 days postflight in order to determine what effect the space flight had on the amount of calcium being absorbed from the diet and being released as a result of bone breakdown. The long-term effects of the space flight on bone turnover were studied by analysis of specimens obtained up to two months after return to Earth.

Analysis accomplished during FY97 was directed toward answering the primary hypotheses of the experiment. The results showed that: 1) bone breakdown increases by about day 4-5 of flight, remains elevated for the duration and for at least 2 weeks postflight; 2) blood ionized calcium increases early in flight, even before the increase in bone breakdown; 3) the hormonal regulatory mechanisms for blood calcium respond the same inflight as on the ground, with a decrease in blood parathyroid hormone in response to the increased serum calcium as

early as 2 hours into flight; and 4) there is no evidence for a decrease in bone formation in adult humans in two weeks of space flight.

During FY96, the majority of data and sample collection was done for the LMS flight and the materials logged and stored at JSC until the specimens could be allocated to the various experimenters. During FY97, specimens were transported from JSC to the investigators' laboratories and analysis begun. We have completed primary analyses of all the serum specimens and pooled urine specimens from the four crewmembers. Preliminary analysis of diet data was begun, but errors in the data collected at JSC prevented these data from being used in final metabolic calculations. Corrected data are to be available from JSC sometime in FY98.

Preliminary results were presented at a National Academy of Sciences panel in June 1997, with more complete results presented at the LMS 1-Year Symposium in August 1997.

In addition to the general results noted in the abstract, there were some unexpected findings which may warrant further investigation. Because of some alterations in preflight sample collection, we obtained our last preflight blood sample the morning of launch, followed by the first in-flight sample about 2-3 hours into flight. Even at that point, serum ionized calcium was elevated and the serum parathyroid hormone concentration was decreased, substantiating our hypothesis that there would be an early increase in blood calcium. However, the time of onset of the increase was earlier than could be explained by the increase in bone resorption noted by days 4-5, and appeared to be related to a very early transient phenomenon. Upon further investigation, we found that the increase was accompanied by a mild metabolic acidosis, corresponding to about a 5% increase in serum H⁺, and a slight decrease in urinary chloride in the few days of light. We believe that there may be changes in renal handling of ions in the first few days of light, and that this effect is superimposed on the bone changes we saw as expected a few days later. One of our efforts in FY98 will be to try to explain this effect and define its origin.

This study addresses a number of issues directly relevant to the study of osteoporosis on Earth. The issues are both technical and scientific. Our efforts to develop microassays capable of making a significant number of measurements on small blood samples can be expanded to the clinical evaluation of patients with osteoporosis, as well as pediatric patients with disorders of calcium metabolism. We have worked for several years to optimize a method to measure calcium absorption and bone calcium turnover using stable calcium isotopes, and this methodology has been refined to the point that we are close to developing a clinical assay for these parameters at a fraction of the thousands of dollars that these tests now cost. This will add significantly to the evaluation of calcium metabolism in patients with osteoporosis and especially in other metabolic disorders of calcium.

One of the major scientific outcomes of our flight study will be the ability to predict, in individuals, the effect of a transient stimulation of bone remodeling on the later status of the skeleton. The long-term effects of repeated short-term exposure to a skeletal stimulus cannot at present be predicted accurately, and the correlated data we will obtain from this study will allow us to develop a model to do this, not only for repeated exposure to space flight, but also to exposure to other factors. The current direction of research into the clinical treatment and prevention of osteoporosis is in the modification of skeletal responses to various stimuli, whether they be pharmacologic or endogenous stimuli. The ability to predict long-term skeletal outcomes from short-term studies would be of tremendous value in evaluation of potential therapies for patients, and especially in individualizing treatment regimens. One aspect of this research which we may be able to address with space flight studies is the possibility that we can modify membrane permeabilities in the body under certain conditions, which would open new areas for research in therapeutics and drug delivery. While this is speculative, it may have application not only in osteoporosis but in oncology, hematology, and other fields.

FY97 Publications, Presentations, and Other Accomplishments:

Arnaud, C.D. and Cann, C.E. "Effects of spaceflight on the skeleton: The NASA experience." National Academy of Sciences Symposium (June, 1997).

Cann, C.E. and Arnaud, C.D. "Effects of spaceflight on calcium metabolism: E074 one-year results." LMS 1-Year Symposium, Montreal, Canada (August, 1997).

Relationship of Long-Term Electromyographic (EMG) Activity and Hormonal Function to Muscle Atrophy and Performance

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Funding:

UPN/Project Identification: 106-30 (E036)

Solicitation: AO-OSSA-84

Initial Funding Date: 1992

Expiration: 1997

Students Funded Under Research: 8

Post-Doctoral Associates: 3

FY 1997 Funding: \$140,000

Flight Information:

Experiment ID: 284036

Flight Assignment: LMS (STS-78, June 1996)

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: TVD

Task Description:

Degradation in skeletal muscle function associated with space flight may be caused, at least partially, by altered motor function. This experiment tests the hypothesis that the inactivity of muscles in space modifies a person's ability to control movement. It also tests the body's ability to secrete chemicals that can protect against muscle atrophy and weakness.

The experiment has four segments: a 24-hour electrical activity (EMG) test, a torque-velocity/motor-control task, a fatigue test, and an endocrine response to exercise activity. The 24-hour EMG test will identify the subject's muscle activity levels during routine activity, measuring electrical impulses through 12 electrodes placed on five muscles on the right leg and arm. Once during each of the three 24-hour tests, each payload crew member will perform movements of the right leg and arm, using the Torque Velocity Dynamometer to determine levels and patterns of EMG activity at maximum and submaximum levels of effort. Also, in this second segment of the experiment, subjects test their ability to apply pressure by compressing a hand-grip dynamometer, a device that measures grip strength. These tests will provide information on the strategies of the nervous system to regulate controlled muscular activity and on how the microgravity environment modifies these neural strategies. The results also may reveal the importance of muscle use in the learning and forgetting of motor skills and may shed light on whether unstressed muscles and their neural networks compensate

appropriately so that they regain the ability to move precisely or to maintain the appropriate postures in Earth's gravity and a microgravity environment.

The effects of space flight on the fatigability of the ankle extensors (calf muscles) will be tested by having crew members perform a series of repetitive submaximal and then maximal isometric contractions. Both the torque of the ankle (force output) and the EMG of the ankle extensors will be measured throughout the fatigue tests. These data will provide an indication of the relative importance of neural, as compared to muscular, fatigue, helping to explain changes in motor performance, as well as how the gravitational environment affects these responses.

The final component of this investigation is designed to test hormonal response to the fatigue test. The hormone of primary interest for this test is growth hormone, which will be measured from venous blood samples taken from the arm. During the mission, the tests will be performed twice, once early in the mission and once toward the end of the mission.

On-orbit results will be compared with pre- and postflight data to determine the effects of microgravity on the level of muscle activity, ability to control muscles, and capacity to secrete growth hormone.

Maintain Constant Torque Test

Supraspinal and proprioceptive inputs to motor pools are altered by microgravity. It was hypothesized that neural control to motor pools of anti-gravity muscles might be affected by alterations of sensors influenced by gravitational forces. To test this hypothesis, the effects of space flight on the ability to maintain constant torque output at the elbow or ankle joint were studied. Four male astronauts were requested to maintain a torque of 10% or 50% of a maximal voluntary contraction (MVC) during 10 degree peak to peak sinusoidal movements at 0.5 Hz and 1 Hz with and without visual feedback of torque output. For elbow flexion at 10% MVC, subjects underestimated torque output relative to preflight values from the first flight day to day eight postflight. No changes in maintaining a requested torque during flight were observed in the other muscle groups. Additionally, rises in agonist EMG activity on the second day postflight were observed. As well, a marked decrease in antagonist muscle activity for most tasks during flight was evident. The results of the present study suggest that the ability to execute a targeted torque was altered during and following space flight, particularly in dynamic tasks involving elbow flexion.

Torque output estimation changes occur in motor coordination and strength in astronauts during and following space flight. Moreover, astronauts have reported that increased effort was required for movements following return to normal gravity environment. It is possible that changes in neuromuscular balances underlying muscle force estimation take place during exposure to microgravity. This study was designed to investigate this question and attempt to measure changes in effort and/or sense of muscle output. Muscle output estimation was examined in four male NASA crew members before, during, and following a 17-day space flight (LMS STS-78 mission). Torque and EMG were recorded while subjects performed isometric muscle contractions at the ankle, elbow, and hand in response to requests for specific muscle output levels (as percentage of maximum). Over the course of the study, maximal muscle torques remained largely unchanged. Muscle output estimation was affected during space flight at lower output levels in some muscles. In elbow extensors, this was evident in all subjects; in plantarflexors, for 3 of 4 subjects; and in dorsiflexors and hand grip force for 2 of 4 subjects. The surprising finding that muscle strength remained intact throughout the flight was attributed to the large amount of exercise performed by the crew members during the LMS mission. Likewise, the relatively small changes seen in muscle output estimation during this mission might have been greater had a less extensive exercise regime been utilized.

Maintenance of Plantarflexor Muscle Function in Humans During a 17-day Space Flight

This part of the experiment investigated the effects of space flight on plantarflexor muscle torque and EMG activity during submaximal voluntary contractions and MVCs. The subjects performed a protocol of unilateral

isometric contractions consisting of 2 "initial" MVCs, 24 contractions at 30% MVC, 2 MVCs, 12 contractions at 80% MVC, and 2 MVCs, all performed at a work:rest ratio of 4:1 sec. Torque and EMG activity are always expressed relative to the torque or EMG activity recorded for the highest of the two MVC requested at the beginning of the Fatigue Test. There was no significant difference in either torque or EMG activity recorded during the "initial" MVCs within or between testing conditions (Baseline vs In-flight vs Recovery; mean +/- SD ranges: 146-176 +/- 6.7-32.3 Nm). However, a significant decrease ($p < 0.05$) occurred for the MVCs performed after both the 30% (94.6 - 103.9% of initial MVC) and 80% (81.8 - 93.9% of initial MVC) MVC series of contractions, but the decreases noted within session were independent of space flight. The MVC's EMG:torque ratio increased ($p < 0.05$) 12% for the SO and 10% for the MG between the maximal contractions performed after the 30% and 80% MVC series, but again there were no space flight effects. Furthermore, there were no changes in the ratios of SO:MG EMG during any of the MVCs. These data suggest that an exercise protocol which induced modest fatigue in a 1-G environment had a similar effect after 13 days of exposure to microgravity as well as upon return to 1-G. The absence of space flight effects, however, could reflect the cumulative volume or patterns of plantarflexor muscle contractions performed for other experiments during flight.

Hormone Response to Exercise

Hormonal responses of four male crew to a muscle fatigue test were studied before (-30 and -12 days), during (2/3 and 13/14 days), and after (+2, +4, +8, and +15 days) 17-days of space flight. The fatigue test involved a series of unilateral isometric plantarflexions and included 6 MVC, 24 contractions at 30% MVC, and 12 contractions at 80% MVC all performed at a 4:1 sec work:rest ratio. Blood was collected prior to and immediately following the fatigue test to measure plasma growth hormone by bioassay (BGH; micrograms · L⁻¹) of tibia epiphyseal cartilage growth in hypophysectomized rats. Before space flight, plasma BGH increased ($P < 0.05$) from pre to post fatigue test [-30 days, 2509 +/- 289 to 5369 +/- 2150; -12 days, 3668 +/- 2546 to 9819 +/- 6575]. During space flight, the BGH response was blunted at 2/3 days [2460 +/- 592 to 2930 +/- 510], and essentially absent by 13/14 days [3039 +/- 306 to 3057 +/- 473]. Following space flight, the BGH response remained absent at +2 days [2379 +/- 788 to 2387 +/- 676], but had returned by +4 days [2392 +/- 427 to 5470 +/- 1889], +8 days [2695 +/- 477 to 5675 +/- 404], and +15 days [2479 +/- 417 to 8837 to 5213]. In conclusion, the exercise-induced release of BGH was inhibited during space flight and early recovery, but had returned to normal by four days after space flight. These results are similar to a previous 17-day bedrest study and suggest that the chronic level of neuromuscular activity/loading regulates the exercise-induced release of BGH, perhaps via a muscle-afferent-pituitary axis.

24-hour EMG

Preliminary data analysis of one crew member's 24-hour EMG data shows a definite increase in muscle activation on flight day (FD) 1 for the SO, TA, BB, and TB muscles. Overall, the SO and TA daily activation increased during flight, whereas a slight decrease in the GM daily level of EMG activity is observed. The TB also appears to be activated to a greater extent throughout flight as compared to the ground based data. Besides the marked increase in BB activation on FD 1, the daily muscle activation estimates computed for FD 7 and 13 are similar to the baseline and recovery values.

Estimation of Elbow and Ankle Joint Position

While most studies of altered standing postural control during and after space flight have emphasized vestibular mechanisms, the present study focused on neuromotor control of the ankle in the absence of weight support. The ability to estimate ankle joint position was tested in four male astronauts before (-30 and -12 days), during (2, 7, and 13 days), and after (+0, +2, +4, +8, and +15 days) a 17-day space flight (STS-78). Subjects were asked to estimate 100 degree or 120 degree joint angles during passive (relaxed) and active (10 percent maximal agonist effort) isokinetic plantarflexion (PF) and dorsiflexion (DF). Subjects also estimated these joint positions during low resistance isotonic PF. For each angle, 6 (isokinetic) and 3 (isotonic) trials were requested in random order. No effects of space flight occurred for any isokinetic condition. For passive isokinetic

movements, angle estimates were most accurate during PF (mean error +3 degrees for 100 degrees and -6 degrees for 120 degrees), whereas mean estimates were 15 degrees slower ($P < 0.05$) for both angles during DF. No differences occurred between passive versus active isokinetic PF or DF. Estimates of angles during isotonic PF (mean error -6 degrees for 100 degrees and -18 degrees for 120 degrees) were less than passive isokinetic PF, but greater than passive isokinetic DF ($P < 0.05$). Additionally, the estimates for 120 degrees during isotonic PF were reduced ($P < 0.05$) on the 2nd and 7th days of flight compared to specific preflight and/or postflight days. These data demonstrate that ankle joint angle estimation was: 1) most accurate during isokinetic PF; 2) not changed by low level agonist muscle exertion during isokinetic movements; and 3) less accurate during space flight than before or after for isotonic PF. These data suggest that the proprioception of ankle position was differentially affected during PF and DF. Further, the microgravity effects were manifested without weight bearing posture.

In addition to the ankle, subjects were also asked to estimate 90 degree and 135 degree elbow joint positions during similar tests. The following conclusions were drawn based on the results:

- 1) In the free moving limb (isotonic), requested joint angles were underestimated and overestimated in the ankle and elbow, respectively.
- 2) The estimate of joint angle is influenced by passive limb movement and whether movement is flexion/extension. When the joint is moved passively during extension, the estimate of joint angle increases for the ankle, but decreases for the elbow. Therefore, they got more accurate. When the joint is moved passively during flexion, the estimate of joint angle is smallest for both elbow and ankle. Therefore, they were underestimating positions for both joints.
- 3) The estimate of joint angle during passive isokinetic movement is not altered by active exertion of the agonist.
- 4) Space flight did not affect the differences in joint estimation observed at 1-G for isotonic versus passive isokinetic flexion/extension or passive versus active isokinetic movements.
- 5) For the ankle, only the isotonic extension condition of 120 degrees requested angle showed a decrease during early and mid-flight.
- 6) Estimates of elbow angles were decreased during space flight and elevated during early recovery for all test conditions, at both requested joint angles (for 135 degrees, some are nonsignificant "trends").

This project has four segments addressing problems related to neuromuscular diseases as well as the problem of muscle atrophy as occurs in response to space flight. Further, these studies contribute to our understanding of the control of movement in the unique space flight environment and has considerable bearing on the control of movement, such as standing and maintaining upright posture in the aging population. The proposed research should give us a considerable clearer understanding of the physiological signals which may contribute to the maintenance of muscle mass. For example, the activity levels in muscles of the arms and legs will be monitored during normal activities at normal gravitational loading as well as in the microgravity environment. These data should indicate the importance of activity in maintaining normal mass and functional properties of flexor and extensor muscles. The role of activity of specific muscles in maintaining normal levels of control of movement will also be determined. One of the major advantages of the proposed experiments in efforts to understand basic biological processes is that the normal neuromuscular system will be studied in an abnormal physiological environment, i.e., the altered function is caused by an altered environment, not by an altered capability of the physiological system being studied as would be the case with surgical or pharmacological manipulation.

Another phase of the proposed experiments addresses a fundamentally new biological process previously undiscovered. We have found that muscle spindle receptors can stimulate or inhibit the release of growth

hormone factors. Further, these receptors seem to become less efficacious with bedrest, and we hypothesize that similar effects will be caused by chronic exposure to space flight.

Each phase of these experiments has important implications on the optimization of rehabilitative care in addressing problems related to neuromuscular dysfunction as well as some aspects of hormonal function. These results could have a fundamental and large impact on currently accepted approaches to the rehabilitation of a number of medical conditions in which a person remains in bed for prolonged periods, in individuals with compromised neuromuscular systems, and in the aging population.

FY97 Publications, Presentations, and Other Accomplishments:

Boorman, G.I., Edgerton, V.R., Hodgson, J.A., Goulet, C., and Garfinkel, A. "Autoscaling of perceived muscle output." (Abstract) *Neurosci. Abstr.*, vol. 22, no. 22, 1637 (1996).

Edgerton, V.R. and Roy, R.R. "Neuromuscular adaptations to actual and simulated spaceflight" in "Handbook of Physiology - Environmental Physiology." pp 721-763 (1996).

McCall, G.E. "Regulation of growth hormone release by muscle afferent activity." UCLA Pediatric Endocrinology Seminar (September 24, 1997).

McCall, G.E., Goulet, C., Boorman, G.I., Hodgson, J.A., Roy, R.R., and Edgerton, V.R. "Maintenance of plantarflexor muscle function in humans during a 17-day spaceflight." (Abstract) *Med. Sci. Sports Exercise*, vol. 29, S28 (1997).

McCall, G.E., Goulet, C., Grindeland, R.E., Hodgson, J.A., Bigbee, A.J., and Edgerton, V.R. "Bedrest prevents release of tibial growth factor but not growth hormone in response to muscle activity." (Abstract) *Neurosci. Abstr.*, vol. 22, no. 2, 1346 (1996).

McCall, G.E., Goulet, C., Grindeland, R.E., Hodgson, J.A., Bigbee, A.J., and Edgerton, V.R. "Bedrest suppresses bioassayable growth hormone release in response to muscle activity." *J. Appl. Physiol.* (In Press).

Effect of Weightlessness on Human Single Muscle Fiber Function

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Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center

Task Description:

This experiment investigates the cellular causes of muscular atrophy and weakness in space. Investigators will establish the extent to which changes in cell function affect skeletal muscle function and performance, as well as the time course for any such changes. The results of assessing the work capacity of individual muscle fibers as well as intact muscle groups will contribute to a better understanding of microgravity-induced muscle atrophy and help refine existing countermeasures against the deleterious effects of weightlessness on human muscle performance. An increased understanding of the cellular processes involved in muscle wasting also may be relevant to scientists concerned with the processes of aging.

Specifically, the science team will study the relation of oxygen consumption ($\dot{V}O_2$) to muscle function and performance. Oxygen uptake and energy expenditure are closely related. When slow-twitch muscles are exercised, they rely primarily on an aerobic process (one requiring oxygen) to extract the energy stored in carbohydrates, fats, and proteins. Fast-twitch fibers are more dependent on energy produced by the anaerobic breakdown of stores of glycogen. If a human's maximal oxygen uptake capacity declines in space, the slow-twitch muscles may not be as efficient because of their increased dependence on anaerobic energy sources.

The experiment has three components: cardiovascular exercise testing, leg muscle (right calf) testing, and muscle biopsy. In the cardiovascular exercise element, investigators will compare preflight, inflight, and postflight measurements of each payload crew member's capacity to take oxygen into the body (the maximum oxygen uptake) to determine any changes in uptake capacity. Muscle testing will evaluate how well the right calf muscles contract and how long they can work before tiring. Finally, scientists will obtain biopsies of crew members' muscle tissue. Physiological and biochemical assays of single fibers isolated from the biopsies will disclose any changes that may have occurred at the cellular level.

The 17-day LMS space flight resulted in significant decline in the whole body aerobic capacity of the crew, and in the functional performance of individual slow-twitch and fast-twitch fibers. The fiber atrophy and decline in function (force and power) was greatest in the slow type I fibers of the antigravity soleus muscle. Considerable subject variability occurred. For example, the decline in peak power of the soleus type I fiber ranged from 40 to 12 %. The reason for the different responsiveness of the crew to microgravity is unknown, but could relate to the degree of exercise countermeasure performed.

Cell atrophy was the primary cause of the decline in fiber force and power as the force per cross-sectional area showed only minimal changes. In the slow type I fibers of the soleus, two of the four crew members showed a significant drop in peak power. All four subjects showed an increased shortening speed, and this partially protected against the loss of power. The increased velocity appears to have resulted from a selective loss of filamentous actin. This increased the actin-myosin filament spacing which in turn allowed a faster cross-bridge cycling rate and increased fiber speed. The structural rearrangement of the filaments represents an important adaptation to preserve cell power. One expects that this strategy is considerably more efficient than switching the myosin phenotype from the slow to the fast isozyme. The latter strategy occurs in rats flown in space and results in a faster but less efficient muscle.

The reduced force and power of the individual fibers of the soleus and gastrocnemius did not result in a compromised calf muscle function. This suggests that the cellular changes were either too small to be detected or that the crew compensated for the decline in cell function by altering their motor unit recruitment pattern. In future studies, it will be important to determine if the deleterious changes in cell function get progressively worse with increasing duration of flight or if a new steady state is obtained. If the latter occurs, we need to know at what point the new steady state is obtained. Animal studies suggest that microgravity induces an altered cell metabolism, such that, skeletal muscle fibers show an increased reliance on carbohydrates and a reduced ability to oxidize fats. In the LMS bed rest study, we observed an increase in the enzymes of carbohydrate metabolism and cell glycogen concentration post bed rest. These data were consistent with an increased reliance on carbohydrate metabolism. In the LMS flight, there was no change in the oxidative enzymes b-hydroxyacyl-CoA dehydrogenase or citrate synthase (CS) except in the slow type I fiber of the soleus where CS significantly increased. In this fiber type, glycogen synthase and glycogen also increased, but the glycogen change was not significant. However, data from others indicate that the crew was in negative caloric balance, and this lack of sufficient caloric intake may have reduced the microgravity induced increase in cell glycogen. We hypothesized that a microgravity-induced decline in fat oxidation might be mediated by inhibition of CoA-carnitine acyl transferase (CAT), the rate limiting enzyme in fat oxidation. However, we observed space flight to have no effect on the activity of CAT in slow and fast gastrocnemius fibers and to increase the activity of this enzyme in the slow type I fiber of the soleus. In future studies, it will be important to determine if fat utilization is inhibited in humans during exercise in space as reliance on cell glycogen and blood glucose coupled with muscle atrophy will lead to an increased fatigability and reduced physical work capacity. Finally, future studies must rigorously test high resistance exercise as a countermeasure to the microgravity-induced cell atrophy.

A major goal of this research is to elucidate the functional changes associated with zero G-induced muscle wasting, and to use this information in the development of effective exercise countermeasures. The program is essential to our ability to explore the universe and work successfully in space. Stated another way, we simply cannot embark on long-term space travel until we can understand and prevent muscle wasting. Similar types of muscle atrophy occur on Earth in various muscle diseases and during the normal aging process. This work will provide an increased understanding of basic muscle function and how it is deleteriously altered with inactivity. We will establish whether the reduced physical work capacity induced by weightlessness is caused primarily by deleterious alterations within the limb skeletal muscles or if a reduced aerobic capacity contributes to the problem. In addition to the direct benefits to space biology, this work will provide the basic knowledge needed for the development of new exercise protocols and strategies that should be more effective than current procedures in slowing the atrophy process associated with aging. Since one of the main problems encountered by older adults is weakness which leads to debilitating falls, these modalities will improve the quality of life and lead to considerable savings in medical costs.

FY97 Publications, Presentations, and Other Accomplishments:

Fitts, R.H., Widrick, J.J., Knuth, S.T., Blaser, C.A., Karhanek, M., Trappe, S.W., Trappe, T.A., and Costill, D.L. "Force-velocity and force-power properties of human muscle fibers after spaceflight." (Abstract) Med. Sci. Sports Exercise, vol. 29, S190 (1997).

Trappe, S.W., Trappe, T.A., Costill, D.L., Lee, G.A., Widrick, J.J., and Fitts, R.H. "Effect of spaceflight on human calf muscle morphology and function." (Abstract) Med. Sci. Sports Exercise, vol. 29, S146 (1997).

Trappe, T.A., Trappe, S.W., Costill, D.L., Lee, G.A., Fitts, R.H., and Widrick, J.J. "Oxygen uptake during submaximal and maximal cycling before, during and after spaceflight." (Abstract) Med. Sci. Sports Exercise, vol. 29, S190 (1997).

Widrick, J.J., Romatowski, J.G., Bain, J.L.W., Trappe, S.W., Trappe, T.A., Thompson, J.L., Costill, D.L., Riley, D.A. and Fitts, R.H. "Effect of 17 days bedrest on peak isometric force and unloaded shortening velocity of human soleus fibers." Am. J. Physiol. (In Press).

Widrick, J.J., Romatowski, J.G., Norenberg, K., Gettleman, G., Trappe S.W., Trappe, T.A., Costill, D.L., and Fitts R.H. "Effect of spaceflight on peak force and maximal shortening velocity of human muscle fibers." (Abstract) Med. Sci. Sport. Exercise, vol. 29, S190 (1997).

Magnetic Resonance Imaging after Exposure to Microgravity

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Flight Information:

Experiment ID: 284054
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center

Task Description:

After the eight-day flight of Spacelab-J, the crew showed evidence of significant atrophy in their calf, thigh, and lower back muscles. This ground-based experiment is designed to document comparable changes in the muscles of the LMS crew during the planned 16-day mission. Using Magnetic Resonance Imaging (MRI) scans, the science team will quantify changes in the volume of individual muscles (soleus, gastrocnemius, quadriceps, hamstrings, adductors, intrinsic low back, and psoas) and will determine the degree and rate of recovery to their preflight states. The MRI scans may demonstrate, for instance, whether the predominantly slow-twitch soleus atrophies faster than the predominantly fast-twitch gastrocnemius. Muscle volume will be compared to muscle performance measurements gathered on orbit during other experiments. Dual photon X-ray absorptiometry, or DEXA, will be used to obtain total body and regional fat and lean tissue mass, which will complement the MRI data. In addition, DEXA will be used to monitor fluid redistribution after flight.

Investigators will also study changes in the cross-sectional areas of intervertebral discs in the lower back; if significant expansion of the disc area is evident, researchers may improve their understanding of the causes of back pain reported by many astronauts. This experiment also will determine any differences in the ratio of fat and water in spinal bone marrow during two weeks in space. These findings may indicate alterations in the ability of the bone marrow to produce new red blood cells.

The project was completed and a final report submitted in September 1997. The most interesting findings of our experiment were the changes in bone marrow T2 following flight. The significance of these findings needs

further work, however we speculate that if these changes represent a response of the trabecular skeleton to loading, measurements such as these may provide an early indicator of bone formation. This information might be useful in assessing the early response to countermeasures against space flight bone loss or to evaluate the effectiveness of treatment for osteoporosis in the elderly. Assuming that alterations that result in bone loss with aging or other causes are at least partially rooted in the bone marrow, these findings could lead to a better understanding of the basic physiology of the remodeling process in various diseases. It may be possible to investigate bone specific changes in remodeling. We would like to verify these findings on another short-term (8-17 days) shuttle mission designed to specifically follow these changes after flight. It would also be interesting to investigate women entering menopause to correlate the ultimate changes in bone mineral density occurring over several years versus the marrow changes.

We did not observe any residual expansion of the intervertebral discs as seen after long duration bed rest; we did observe some slight contraction in the discs following flight which might be important after long duration weightlessness.

As expected, our MRI measurements demonstrated decreases in muscle volume, 3 - 12%, in the calf, thigh and back similar to the changes after 17 days of bed rest. There was fluid movement into the lower limb as evidenced by MRI volume changes between R+0 and R+2 and increases in T2 on R+2 and in some cases on both R+2 and R+10 days. Our data suggest that reambulation after flight or bed rest causes swelling of muscle that lasts several weeks and is probably associated with muscle damage and/or repair.

There were no significant changes in total body BMD or lean tissue after flight, but there appeared to be loss in total body fat which paralleled changes in total body weight.

Space flight measurements have documented that significant bone and muscle atrophy occurs during weightlessness. Knowledge of the extent and temporal relationships of these changes in the individual bones and muscles is important for the development of effective countermeasures. The losses during space flight are believed to result from the reduced forces on the musculoskeletal system. Analogous to space flight, inactivity in 1-G will cause bone and muscle loss. The loss of bone and muscle with aging occurs in both men and women, resulting in a significant public health problem. Although the exact cause of bone and muscle loss with aging is not understood, one important risk factor is disuse. Men and women become less active as they grow older, and that may play an important role in the elderly and in patients immobilized for medical reasons. In addition, muscle atrophy is an important component of many disease states as well as aging; therefore understanding the role of disuse versus other causes is important for elucidating the physiological mechanisms of muscle atrophy. The relationship of muscle atrophy to muscle performance is not well understood. The LMS flight will examine decrements in muscle performance with measurements of muscle specific atrophy.

Back pain is a common health problem. There are several causes for this complaint and it often involves the intervertebral discs. Bedrest is frequently recommended as a component of patient management. Our studies demonstrated that overnight or longer bed rest causes expansion of the disc area, reaching an equilibrium value of about 22% (range 10 - 40%) above baseline. In space, where the external mechanical loads are greatly reduced, the disc probably expands significantly. These changes which are rapidly reversible after short-duration flights, may be an important consideration during and after long-duration missions or bedrest on Earth, e.g., disc physiology may be altered. Also, this change in the disc size may be causally related to the back pain experienced during space flight.

FY97 Publications, Presentations, and Other Accomplishments:

LeBlanc, A., Lin, C., Evans, H., Rowe, R., Shackelford, L., and Hedricks, T. "Magnetic resonance imaging after exposure to microgravity." LMS One Year Science Review, Montreal, Canada (August 20 - 21, 1997).

LeBlanc, A., Lin, C., Evans, H., Shackelford, L., West, S., and Hedricks, T. "T2 vertebral bone marrow changes after space flight." American Society for Bone Mineral Research, Cincinnati, OH (September 10 - 14, 1997).

LeBlanc, A., Lin, C., Evans, H., Shackelford, L., West, S., and Hedricks, T. "Vertebral bone marrow changes following space flight." 12th Man In Space Symposium, Washington, DC (June 8 - 13, 1997).

Lignin Formation and Effects of Microgravity: A New Approach

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Flight Information:

Experiment ID: 9307247
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: PGU

Task Description:

The focus of the plant science experiment on the Life and Microgravity Spacelab mission is to establish the effect of the microgravity environment on the ability of plants to form a reinforcement tissue known as reaction wood. On Earth, woody plants produce this distinctive reinforcement tissue when their stems are bent contrary to their normal orientation. The reaction wood formation helps restore the stem to its upright position, which contributes to the plant's survival, but has an adverse effect on wood quality and texture.

Conifer seedlings will be placed in the Plant Growth Unit (PGU) in an orientation that favors reaction wood formation in Earth's gravity. The crew will perform a daily status check of PGU systems, photograph the Plant Growth Chambers, and fix some of the plants, effectively stopping their growth and development at predetermined intervals during the mission. Two of the six chambers will be opened for plant fixation, at which time the plants will be harvested, chemically fixed, and frozen for postflight analysis. Electron and light microscopic study of the samples will define the time and place of reaction wood formation and the extent to which it forms. Chemical and biochemical analysis will complement the study, enabling scientists to measure the effects of microgravity and reaction wood formation and, if possible, to define the regulatory enzymes and genes involved. The technology used for this experiment will be incorporated into future space station facilities for plant growth.

The Progress Report for FY97 covers the time-frame from October 1, 1996 to September 30, 1997. The two main objectives of this currently funded NASA grant include (i) to determine whether compression wood formation occurs in microgravity, and to establish the time-course of its [potential] induction at both 1-G and in

microgravity, respectively, and (ii) to develop methodology to determine the rate-limiting step(s) involved in formation of the monomeric lignin precursors from phenylalanine, including how the corresponding monolignols are transported to the plasma membrane and how the various metabolites and enzymes are organized at the subcellular level.

Progress to both goals, in this fundamentally important area of gravitational plant biology, are well in hand and proceeding smoothly. Individual progress to each objective is described below.

STS 78 Mission: Shuttle Columbia was launched on June 22, 1996, from Kennedy Space Center, with the N.G. Lewis team being present at both KSC and Dryden (in case of a California landing and recovery). The space flight experiment, while answering the question of whether compression wood formation occurred under such conditions, was not without difficulty. Although the bending experiments were initiated in space, the shuttle cabin temperatures were higher than any recorded previously ($> 29^{\circ}\text{C}$), which on average had a deleterious effect on the growth of several specimens. Nevertheless, those still in obviously good conditions (i.e., containing new growth) were harvested, sectioned, and fixed both in space (at days 10 and 13), with the remainder harvested, sectioned and fixed upon recovery after the 17-day flight. The results obtained are as follows. Under the conditions employed, both sets of plants (i.e., microgravity and 1-G grown) were essentially identical, all forming compression wood when orientated at 45° . On the other hand, compression wood formation did not occur when either plants were placed in a vertical configuration. Note also that the experiments had to be repeated several times post-flight (under flight conditions) to verify that the difficulties experienced were, in fact, due to high temperature. This was established to be the case (data not shown).

Most authorities might have expected compression wood not to be formed if the gravitational vector was removed. Thus, to account for its formation in space, either the microgravity influence is still large enough to ensure that the organisms can still respond to it, or more likely, the effect of mechanical loading overrides the gravitactic responses (i.e., due to overlapping signal transduction, perception, and response mechanisms). Put in another way, even in microgravity, the plants can make appropriate corrections to alleviate the stress gradient introduced by bending, thereby forming compression wood.

Work is also currently in progress, using a freeze-fracture approach, to examine the cellulose microfibril orientation of the space flight plant tissues, in order to determine if the effects on cell wall organization were altered in microgravity in either the newly formed compression wood or normal xylem cells.

Metabolic Flux: The question of metabolic flux was investigated in terms of post-phenylalanine metabolites into all possible derivatives, which accumulated either intra- or extra-cellularly in *P. taeda* cells and cell media. *P. taeda* cells were induced to biosynthesize the monolignols, *E*-coniferyl and *E*-*p*-coumaryl alcohol, as before, by treatment with 8% sucrose in the presence of 20 mM KI. Different levels of phenylalanine were then exogenously supplied (from 0 to 40 mM, in 5 mM increments) in order to ensure that the induced pathway was not substrate-limited.

Several critically important findings were made by both isolating and quantifying the levels of *all* metabolites from phenylalanine to the monolignols. Metabolites were measured, both intra- and extracellularly, at different time intervals over the time-frame of 0 to 48 hours. First, only trace levels of the CoA esters, the corresponding aldehydes and monolignols were noted intracellularly, with no glycosides of the monolignols being observed. On the other hand, essentially only three metabolites, *E*-cinnamic, *E*-*p*-coumaric, and *E*-caffeic acids, built up in the cells, whose amounts varied depending upon phenylalanine availability. Over a 12 hour time period, for example, the levels of *E*-cinnamic acid increased more than 10 fold [at 10 mM phenylalanine], but then dropped off to only a two-fold increase [at 40 mM phenylalanine]. *E*-*p*-coumaric acid levels behaved in a similar manner, with increases of 10 fold [at 10 mM phenylalanine] being noted at 12 h, but this decreased to about 20% if 40 mM phenylalanine was added. Levels of *E*-caffeic acid, on the other hand, increased four-fold [at 10 mM phenylalanine], but then also reduced markedly at higher phenylalanine concentrations. By contrast, no changes in the trace levels of intracellular monolignols or other metabolites were noted. Thus, these initial metabolic flux experiments revealed unambiguously that the regulatory steps in lignin biosynthesis involve not

only phenylalanine ammonia lyase, but the effects of different phenylalanine concentrations, and the subsequent metabolism of the corresponding cinnamic acids, i.e., which represent "slow" or "limiting" steps for further conversion into monolignols.

In a related study, we have systematically varied the time of subculturing, and phenylalanine, sucrose and KI concentrations. We have identified conditions which resulted in the very significant discovery of a metabolic switch, whereby *E-p*-coumaryl alcohol formation is switched to that of *E-coniferyl* alcohol synthesis, and vice versa. These results now set the stage to comprehensively define, for the first time, how the pathway is truly regulated and how selective monolignol formation is controlled. As indicated above, our results reveal more complex interactions than just a single "rate-limiting step or steps." Instead, the integration of differing metabolite levels (including potential enhancers and feedback inhibitors), variable enzymes activities (induced and non-induced) and differing transcript levels are presumed to be involved. These will be defined in the coming months.

In this context, we are also establishing the subcellular organization of both metabolites and enzymes involved in the post-phenylalanine pathway. Conditions have now been defined for organelle fractionation (vacuoles, ER, Golgi, etc.) which are being assayed for specific enzyme involvement. Additionally, vesicles are being isolated in order to prove unambiguously the nature of the monomer(s) undergoing transport into the cell wall. This is necessary in order to obtain a comprehensive view of the lignification process.

The emphasis of the laboratory is to understand how wood formation can be biotechnologically exploited. Recent work has identified the first three genes involved in heartwood formation. This is an important discovery since heartwood utilization for lumber, pulp, and paper production represents an approximately 135 billion dollar industry per annum. Similar genes are also involved in dietary fiber conferring chemoprevention against breast and prostate cancer in dietary fibers. All genes and potential applications are currently being patented, with provisional patents filed one year ago.

FY97 Publications, Presentations, and Other Accomplishments:

Bernards, M.A. and Lewis, N.G. "The macromolecular aromatic domain of suberin: A changing paradigm." *Phytochemistry* (In Press).

Bernards, M.A. and Lewis, N.G. "Induced phenylpropanoid metabolism in suberized potato tubers." Annual Meeting of The American Society of Plant Physiologists. Vancouver, British Columbia, Canada (August, 1997).

Costa, M.A., Gang, D.A., Wang, H.-B., Halls, S.C., Davin, L.B., and Lewis, N.G. "Overexpression of recombinant dirigent protein conferring specificity in monolignol coupling." Annual Meeting of The American Society of Plant Physiologists. Vancouver, British Columbia, Canada (August, 1997).

Croteau, R., Kutchan, T.M., and Lewis, N.G. "Secondary metabolites" in "Biochemistry and Molecular Biology of Plants." Edited by: Buchanan, B.B., Gruissem, W., and Jones, R.L. American Society of Plant Physiologists (In Press).

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Davin, L.B., Wang, H.-B., Crowell, A.L., Bedgar, D.L., Martin, D.M., Sarkanen, S., and Lewis, N.G. "Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center." *Science*, 275, 362-366 (1997).

Davin, L.B., Xia, Z.-Q., Costa, M.A., Fujita, M., and Lewis, N.G. "Purification of secoisolariciresinol dehydrogenase." Annual Meeting of The American Society of Plant Physiologists. Vancouver, British Columbia, Canada (August, 1997).

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- Gang, D.R., Dinkova-Kostova, A.T., Davin, L.B., and Lewis, N.G. "Phylogenetic links in plant defense systems: Lignans, isoflavonoids and their reductases" in "Phytochemical Pest Control Agents." Edited by: Hedin, P. ACS Symposium Series. American Chemical Society, Washington, DC, 658, pp 58-89 (1997).
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- Gang, D.R., Fujita, M., Davin, L.B., and Lewis, N.G. "Lignans in cancer prevention/treatment and in plant defense." Annual Meeting of The American Society of Plant Physiologists. Vancouver, British Columbia, Canada (August, 1997).
- Kato, M.J., Chu, A. Davin, L.B., and Lewis, N.G. "Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds." *Phytochemistry* (In Press).
- Lewis, N.G. US-Japan Binational Biosynthesis Seminar, Wintrop, WA (June, 1997).
- Lewis, N.G. "The evolution of land plants and the phenylpropanoid pathway." Symposium on Information Processing Systems in Plants: Their Evolution and Function. Davis, CA (March, 1997).
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- Marchand, P.A., Kato, M.J., and Lewis, N.G. "Oxygen insertion in *Sesamum indicum* furanofuran lignans. Isolation and hemisynthesis of (+)-sesamone." *J. Nat. Prod.* (In Press).
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Human Sleep, Circadian Rhythms and Performance in Space

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FY 1997 Funding: \$ 110,000

Solicitation: 89-OSSA-13
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Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 8913048
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center

Task Description:

This was the first simultaneous study of sleep, circadian rhythms, and task performance of a group of astronauts in response to a microgravity environment. The experiment evaluated effects caused by microgravity and by the absence of terrestrial time cues (zeitgebers) and normal social contacts. Scientists hypothesized that the severe weakening of social and physical zeitgebers during the mission and/or unusual conditions within the environment (microgravity, cramped conditions, and stress) would disturb circadian rhythms which, in turn, would lead to poorer sleep and degraded task performance. Results may help explain challenges to the biological clock that occur on Earth as a result of shift work and jet-lag.

For two 72-hour periods, each of the payload crew members wore a special belt pack connected to a sleep cap with 10 electrodes attached to the head. The system provided data about brain waves (electroencephalography), eye movements (electro-oculography), and muscle tone (electromyography) while the crew member was sleeping. These data allowed scientists to categorize each minute of sleep by various types and depths. During the 72 hours, another belt pack recorder received a signal from a temperature sensor indicating the crew member's core body temperature every six minutes. Circadian rhythms were evaluated by measuring urine electrolyte and hormone concentrations at each voiding, by mood and activation testing every two hours during the wake cycle, and by performance testing before each meal. Crew members kept a diary to record sleep quality and alertness on awakening and answered end-of-shift questionnaires to evaluate workload, perceived effort, and fatigue. Except for the urine sampling, sleep data (polysomnography), and core body temperature sampling procedures, all aspects of the protocol used the Payload and General Support Computer. Data was compared with pre- and post-flight tests on Earth. Also, an identical ground study may be performed after the mission under the direction of Dr. Alexander Gundel of the Institute of Aerospace Medicine in Cologne, Germany.

All data analysis has been completed and the final research report for this experiment submitted to NASA. The scientists were very pleased with the amount and quality of data received from the four astronauts that participated in the experiment. From the results of this experiment we have concluded that when careful steps are taken to ensure that the astronauts' work/rest schedule does not lead to circadian desynchrony, there is no evidence that microgravity *per se* will disrupt the human circadian timekeeping system, or that such rhythms will degrade over a 17-day mission. In particular, there was no evidence of amplitude reduction, phase lability or "free-running" behavior. Despite the astronauts' circadian rhythms being intact, there were some effects on sleep, notably a reduction in sleep duration, and a suppression of the deepest stages of sleep (Stages 3 and 4). However, there were no other consistent effects of flight in sleep architecture. The only systematic effect on mood and performance appeared to be an increase in alertness in the early flight measurement block. End of shift questionnaires revealed timeline problems and equipment malfunction to be the major impacts on the astronauts' work.

Life on Earth has developed to be in tune with the cycles of daylight and darkness that stem from our planet's 24-hour rotation. Like most other animals, human beings have a biological clock inside the brain which acts as a timekeeper. For diurnal creatures like ourselves, the clock prepares the body and mind for restful sleep at night and active wakefulness during the day. This clock is referred to as the "circadian system" (Latin: *circa dies* - about a day) because the cycles it generates have a period length that is not exactly 24 hours, but is faster or slower than that figure. For example, for humans, the figure is about 24.3 -25.0 hours, depending on the individual. This means that the circadian system requires time cues or zeitgebers (German: time giver) from the environment in order to keep it exactly in tune with the 24-hour rotation of the Earth.

Night workers and people who travel rapidly across time zones run into problems that arise from their circadian systems. Sleep is often interrupted or shortened, and daytime mood, alertness, and performance are often impaired. Study of sleep, circadian rhythms, and performance in space allows us to understand what happens to people when they are removed from most of the time cues on Earth. Findings from our experiment will thus help us to understand the actions of zeitgebers on the human circadian system, and will help us in providing useful coping strategies to night workers and those suffering from jet-lag.

FY97 Publications, Presentations, and Other Accomplishments:

Carrier, J. and Monk, T.H. "Estimating the endogenous circadian temperature rhythm without keeping people awake." *J. Biol. Rhythms*, vol. 12, 266-277 (1997).

Monk, T.H. and Carrier, J. "Speed of mental processing in the middle of the night." *Sleep*, vol. 20, 399-401 (1997).

Monk, T.H., Buysse, D.J., Billy, B.D., Kennedy, K.S., and Kupfer, D.J. "The effects on human sleep and circadian rhythms of 17 days of continuous bedrest in the absence of daylight." *Sleep* (In Press).

Monk, T.H., Buysse, D.J., Reynolds, C.F., Berga, S.L., Jarret, D.B., Begley, A.E., and Kupfer, D.J. "Circadian rhythms in human performance and mood under constant conditions." *J. Sleep Res.*, vol. 6, 9-18 (1997).

Canal and Otolith Integration Studies (COIS)

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Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9307570
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center

Task Description:

The research protocols in the Canal and Otolith Integration Studies are designed to investigate changes in the central processing of visual and vestibular information necessary for spatial orientation, specifically for gaze control, following adaptation to space flight. The coordination of the vestibulo-ocular reflex, smooth pursuit and saccades for maintaining gaze during combined head and eye tracking will be examined in both pitch and yaw planes. Changes in spatial orientation from a gravitational to a body frame of reference will be studied by quantifying optokinetic cross-coupling with a tilted (oblique) stimulus, and with a horizontal stimulus and head tilt relative to spatial vertical.

As originally proposed, the basic premise of this investigation rests on four points: (1) there is a normal synergy or interaction in the vestibular system pathways between activity arising in the semicircular canals, the otolith organs, the visual system, somatosensory receptors, and probably other sensory systems. Through coordination of the many inputs, the sensation of movement and accuracy of compensatory responses to various states of motion is maintained; (2) otolith input is altered during space flight, i.e., spontaneous activity from the otolith organs associated with signaling position in a gravitational field must be modified as a new set point is established; (3) adaptation will occur in microgravity with corresponding modifications of sensory and motor reflexes until new and appropriate response patterns are established; and (4) in the immediate postflight period, responses will reflect the nature and degree of the inflight adaptation.

Based on these four points, our inclusive hypothesis predicts that during space flight, there will be a modification of the normal synergy that exists to coordinate canal, otolith, proprioceptive, and other sensory input. The first part of this investigation was completed during the STS-42 mission Microgravity Vestibular Investigations (MVI) using passive rotational stimuli. The goal of this research is to complete the MVI scientific objectives as they were originally proposed related to visual-vestibular contributions to active goal-directed spatial orientation tasks.

Status of Data Analysis

- Quantitative data analysis is complete. Data interpretation and statistical analysis is nearing completion.

Preliminary Research Findings

- Postflight testing showed what appears to be significant changes in the pursuit eye movement system (to the point that smooth pursuit eye movements were not present); due to the long duration of the flight, effects observed in the smooth pursuit system lasted longer than anticipated.
- Inspection of the data from the optokinetic eye movement experiment showed strong interactions with head position and gravity during the postflight tests. Hypothesized, but never observed before, was a trend to switch eye movements from the horizontal plane (side-to-side eye movements) for eye movements in the vertical lane (up and down eye movements) when the body was tilted from the vertical position. It was also interesting to note that the crew members during postflight testing consistently over estimated their angle of tilt.
- Due to the complexity of the analysis, no preliminary conclusions could be drawn from the sinusoidal head oscillations portion of the experiment. Analysis is complete.

This experiment is a follow-on set of studies first performed as a part of the MVI flown on IML-1. The hardware required to support this experiment (unlike that for MVI) requires that head and eye movements be measured during goal-oriented tasks in a freely moving subject. This task, once thought to be almost impossible, has been accomplished. The primary benefit will be a new more meaningful way of testing clinical patients. Currently most visual-vestibular testing in the hospital is done in only the yaw axis in a restrained subject. Both the new hardware and methods (along with the baseline data) developed for this experiment promise to initiate a new science, and completely modify the way patients are evaluated.

Aside from the clinical aspects, the benefit to NASA will be the first collection of integrated vestibular and visual data ever collected on shuttle flights of 16 days. This data is extremely valuable in assisting NASA advance to space station flights, and to assist in helping ensure the safety, health, and well being of future astronauts.

FY97 Publications, Presentations, and Other Accomplishments:

Clément, G., Wood, S.J., and Reschke, M.F. "Effects of weightlessness on the spatial orientation of visually induced eye movements." 12th Man in Space Symposium, Washington, DC (June, 1997).

Microgravity Effects on Standardized Cognitive Performance Measures

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Funding:

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Students Funded Under Research: 1 Post-Doctoral Associates: 0
FY 1997 Funding: \$120,000
Joint Agency Participation: DoD

Flight Information:

Experiment ID: 8913063
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: PAWS (laptop computer, track ball)

Task Description:

The purpose of this experiment is to determine the effects of microgravity and fatigue upon cognitive skills critical to the success of operational tasks in space. The Performance Assessment Workstation (PAWS) was developed and validated for space flight to display and collect cognitive performance test data. The performance tests were selected from the DOD Unified Tri-Service Cognitive Assessment Battery (UTC-PAB). The tests measure short-term memory, spatial processing, attention, tracking, and dual timesharing. After orientation training, astronaut performance is compared with pre-flight baselines, in-orbit, and recovery periods for any changes. Measures of cognitive performance created for use in microgravity can assist in the identification of specific cognitive functions responsible for reduced productivity, job satisfaction, and any increases in errors that may lead to accidents. Productivity and safety can be enhanced through the systematic feedback of objective measures of performance of space-based workers to ground-based mission managers and medical monitoring teams. This approach to understanding the performance impact of combined stressors and protecting individuals from their consequences is readily applied both in space and on Earth.

An initial analysis has been completed from a total of 1184 data sets from LMS flight using the PAWS. Four astronauts completed 37 sessions of a 20-minute battery of six cognitive performance tests, a mood scale, and a fatigue rating on a laptop computer. Twenty-four sessions were preflight, 9 sessions were in-orbit, and 4 sessions were postflight. Mathematical models were fit to each subject's preflight data for each of the 14 dependent variables. Expected values were then generated from the models for in-orbit performance. The

mathematical models of learning allowed the assessment of in-orbit effects while removing the expected, small amount of continued performance improvement. The models were linearized to allow the computation of a 95% confidence interval for in-orbit predicted values. Using single subject designs, two astronauts showed statistically significant in-orbit effects. One astronaut was degraded on all three dependent measures in the mathematical processing portion of the Directed Attention test that requires rapid switching from a spatial orientation task to a numerical task involving adding and subtracting a series of numbers. However, the same subject exceeded predicted performance on a compensatory tracking task. The other astronaut was degraded on two dependent measures of the same test (Directed Attention - Mathematical Processing) and on reaction time in the spatial processing Matrix test. The next step is to complete analysis of the mood and fatigue data. Integration of these data with those of an earlier mission (IML-2) will allow a comparative analyses that may explain the performance degradations.

This research seeks to uncover the effects of microgravity on cognitive performance using each subject as his own control. To accomplish this, the effects of other variables such as fatigue must be isolated and independently measured. Similar problems arise in attempting to disentangle the effects of other stressors on Earth from fatigue. The single-subject, performance-modeling approach used in the PAWS experiment has application to similar research problems on Earth. Once baseline cognitive performance is established in an individual, deviations from it can be attributed to the isolated stressors affecting that individual. A performance test can be used to determine how long the individual can work effectively in the stressful environment and how much rest is necessary for recovery. This approach to understanding the performance impact of stressors and protecting individuals from them is readily applied both in space and on Earth.

Once a method exists to assess performance, countermeasures to the stressors can be tested for their efficacy in ameliorating any performance degradation encountered. For example, if work/rest schedule manipulations are causing performance decrements, then less stressful schedules can be designed to increase productivity and reduce the chance of human error in-orbit. This research attempts to objectively demonstrate a method to measure cognitive performance in microgravity. If disruption is discovered, then an attempt to understand the cause of the disruption can be initiated. Unlike that of muscle and bone tissue, performance degradation of the brain can not be attributed to lack of use. Other biological processes will have to be investigated.

This research proposes to isolate the conditions causing cognitive performance degradation. Some of these will be the same as those found on Earth such as lack of sleep, work/rest schedule changes that are too aggressive, use of performance disruptive medications, and excessive task demands. The space environment adds to this list of stressors: confinement, isolation, and microgravity. Only by isolating each of these conditions can the effects of the in-orbit stressors be identified and quantified.

Measures of cognitive performance created for use in microgravity can be applied to the average person on Earth to identify and counter the conditions responsible for reductions of productivity and job satisfaction and increases in accidents and errors. Objective measures of performance can help to focus managers and workers on the conditions leading to optimal work performance and productivity. Space station workers can look forward to realistic work/rest schedules that maximize productivity and job satisfaction while minimizing the chance of work-related accidents and human error. Decisions can be based on objective cognitive performance measures rather than subject judgments that are somewhat independent of productivity.

Measurement of Energy Expenditures during Spaceflight Using the Doubly Labeled Water Method

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Flight Information:

Flight Assignment: LMS (STS-78, June 1996)
 Responsible NASA Center: Johnson Space Center

Task Description:

Previous manned space flight missions have indicated that astronauts may be in negative energy balance during spaceflight. The objectives of this project are to: (1) measure human energy expenditure during flight on the space shuttle and (2) determine whether astronauts are in negative energy balance during space flight. The experiment was conducted on the recent LMS mission. The doubly labeled water method (DLW, $^2\text{H}_2^{18}\text{O}$) was used to measure energy expenditure.

Energy intake was calculated from dietary records. Energy balance will be calculated from both the difference between intake and expenditure and changes in body composition. Energy expenditure was measured over blocks of 6 days. There were two consecutive 6 day blocks preflight (days L-15 to L-9 and L-9 to L-3), two similar consecutive 6 day blocks inflight (days FD-3 to FD-9 and FD-9 to FD-15) and two blocks post flight (R+3 to R+9 and R+9 to R+15). Dietary intake and nitrogen balance were measured for the duration (pre, post, and inflight) of the study. Body fat was determined from both the ^{18}O space and by DEXA.

Four subjects (payload crew) were dosed with $^2\text{H}_2^{18}\text{O}$ for the energy expenditure measurements. To further minimize any problems from variation in background water enrichment during the flight, high doses of $^2\text{H}_2^{18}\text{O}$ (0.35 g kg ^{18}O , 0.15 g/kg ^2H) was given to the four test crew persons. Saliva was used to sample the body water pool. Preliminary results showed: (i) dietary intake was reduced during the flight period to 23.6 + 2.0 kcal. kg $^{-1}$.d $^{-1}$ and (ii) the four payload crew persons were in negative energy balance of 6.1 + 1.7 kcal. kg $^{-1}$.d $^{-1}$ inflight.

This experiment is the first attempt to measure the relationships between energy needs and dietary intake during space flight. The determination of human energy requirements in the microgravity environment is crucial to the designing of life support systems and the accurate assessment of a person's ability to live and work productively

in weightlessness. Available evidence is conflicting. Some studies suggest an increase in energy output during space flight, while others indicate a decrease. Two consequences of a negative energy balance on Earth are the wasting of body protein (especially skeletal muscle) and the depletion of stored body fat. The protein loss may result in muscle weakness leading to impaired performance, increased susceptibility to disease, and delayed healing of wounds. The ability of the individual to function adequately during the critical phases of re-entry and landing may also be impacted.

The objectives of this experiment were: (1) To measure human energy expenditure during flight on the space shuttle by using the doubly labeled water method; (2) To determine whether astronauts are in negative energy balance during space flight on the shuttle; (3) To compare energy expenditure before, during, and after space flight; and (4) To compare energy expenditure during space flight against that found with bedrest. The DLW method was used to measure energy expenditure. This method is a highly accurate means of measuring energy expenditure in a safe, time-efficient manner using only urine or saliva specimens for analysis. Water labeled with the non-radioactive isotopes deuterium (^2H) and oxygen (^{18}O) is ingested by the payload crew. The two isotopes leave the body at different rates. Deuterium leaves primarily in urine while ^{18}O leaves in both water and exhaled carbon dioxide (CO_2). The difference in loss rates is equal to the rate of CO_2 production, which is directly related to the rate of energy expenditure. Energy intake was calculated from dietary records.

The experiment was conducted on the four payload crew persons of the 1996 LMS mission. Crew members monitored their dietary and drug intake, kept a daily activity log, and measured their body mass for the duration of the mission. Energy balance was determined from the difference in energy intake as measured by the dietary log and actual energy expenditure as measured by the DLW method. Comparison of the inflight data against the combination of the preflight and matched bedrest data will indicate whether the energy costs of living and working in space are greater or less than those on Earth for comparable activity.

Although data and sample analysis is still in progress, some of the key results are available and these are summarized below. Dietary intake was successfully monitored on the four payload crew members for the preflight, inflight, and post flight periods. Three of the four crew members lost weight during the mission ($84.6 + 2.2$ vs $81.5 + 3.3$ kg, $p < 0.07$). Nitrogen balance was $21.3 + 6$ mg $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ preflight vs $-37.0 + 4$ mg N $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ($p < 0.05$). Post flight N balance recovered to $24.7 + 14.7$ mg N $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$.

The mean energy intake for the two inflight energy expenditure determination periods was $23.6 + 2.0$ kcal $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Energy expenditure for this period was $29.5 + 0.9$ kcal $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. The difference between intake and expenditure, $6.1 + 1.7$ kcal $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, was statistically significant ($p < 0.05$). Although these values are from the preliminary analyses of the data and may change as we refine the data set, we do not expect any gross changes. The inference is therefore that the crew members were in negative energy balance during the inflight period.

This project will eventually provide information on the relationship between muscle loss, energy expenditure, and activity. While the space flight related muscle loss is likely to affect only a few astronauts, the muscle wasting associated with bed rest is a serious clinical problem, with the elderly being particularly impacted. Thus, the information derived from this study will have direct applicability to a problem that affects a sizable proportion of the American people and is associated with substantial costs.

FY97 Publications, Presentations, and Other Accomplishments:

Stein, T.P. and Schluter, M.D. "Human skeletal muscle protein breakdown during space flight." *Am. J. Physiol.*, vol. 272, E688-E695 (1997).

Extended Studies of Pulmonary Function in Weightlessness

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Funding:

UPN/Project Identification: 106-30 (E030)
Initial Funding Date: 1992
Students Funded Under Research: 1
FY 1997 Funding: \$552,000

Solicitation: AO-OSSA-84
Expiration: 1997
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 284030
Flight Assignment: LMS (STS-78, June 1996)
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: ALFE/GASMAP/LSLE Micro-2

Task Description:

This investigation extends the studies of the human lung in four major areas. Investigators will study lung function after the stress imposed by heavy exercise in the microgravity environment; they will monitor the motion in the rib cage and abdomen to study the effects of microgravity on the musculoskeletal aspects of breathing during rest, during heavy exercise, and during deep breathing; they will make the first measurements in microgravity of the body's response to inhaled carbon dioxide, a response that may be altered by space flight; and they will continue and build on previous studies of how gas is distributed within the lung. Data will be collected four times before the flight, several times during flight, and five times in the two weeks following the mission to provide a comparison with lung function on Earth.

A sequence of breathing tests will measure the concentrations and volumes of inhaled and exhaled gases before and after exercise several times throughout the LMS mission. The data will be stored onboard and downlinked simultaneously to the ground, allowing for interaction between the crew and the investigators. The Astronaut Lung Function Experiment (ALFE) hardware developed for SLS-1 and -2 has been modified and will be used with the addition of the Gas Analysis System for Metabolic Analysis Physiology mass spectrometer and microcomputer. Each crew member will have an individual ALFE personal stowage kit, which consists of a mouthpiece and nose clip. The crew member breathes in either the ambient air of the Spacelab cabin or one of the test gases, depending on the activity being performed and the measurement being sought. Expired gases are continuously monitored while being directed either into the cabin, into the rebreathing bag, or into an exhaust bag. The Belgian-built Respirace suit, a vest-like garment equipped with electronics connected to respiratory transducers located at the chest level and at the abdomen, will be used for the rib cage/chest motion studies.

During FY 1997, work has focussed on data analysis following flight. LMS activities precluded work on many of the preflight tasks associated with Neurolab. As a consequence, LMS data analysis has been delayed considerably by Neurolab activities. A request for a one year, no-cost extension to the LMS contract was submitted and approved, allowing an extra 12 months of time for data analysis activities to be completed. Data analysis is ongoing.

The knowledge gained from the flight program will further the basic knowledge of how the human pulmonary system functions. On this mission, we will extend our previous studies to the areas of musculoskeletal function by studying rib cage and abdominal motion, the effect of heavy exercise on the lung in microgravity, and the changes in the carbon dioxide control signals of ventilation.

The bedrest study will provide a useful set of data on the effect of long-term bedrest on those aspects of pulmonary function. Since many people are confined to bed for long periods of time, this information should have direct benefit to such a group.

Development of the Fish Medaka in Microgravity

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Funding:

UPN/Project Identification: 106-30-32

Solicitation: unsolicited

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$195,938

Flight Information:

Experiment ID: 9401617

Flight Assignment: LMS (STS-78, June 1996)

Responsible NASA Center: Ames Research Center

Task Description:

The Life and Microgravity Spacelab (LMS) Space Tissue Loss-B (STL-B) hardware is being used to test the hypothesis that gravity is required for normal embryo development. Investigators will conduct systematic evaluation of vertebrate development and growth using the fish *Medaka* as a model. The *Medaka* is particularly suited to this experiment since it is a hardy fish whose embryos tolerate reduced temperatures well, allowing researchers to subject the embryos to low temperatures and slow embryonic development. This provides more time to study each stage of vertebrate development and maximizes the effects of microgravity on each stage. Also, the embryos are optically clear, which allows investigators to visually examine molecular markers and the development of the internal organ systems with the STL-B video system.

Concomitant ground-based studies have focused on understanding the effects of slowing down the rate of development, on developing optimal fixation and embedding procedures for maximizing future data return, and on studying the expression of a development-regulating gene during embryogenesis in the *Medaka*.

Flight Studies:

LMS: These studies were undertaken in collaboration with Dr. William Wiesmann and colleagues at WRAIR. The development of flight certified scientific hardware necessary to address the initial questions being asked by embryologists on the effects of microgravity on embryonic development has been reported elsewhere (Weismann, W.P., L.A. Pranger, E.S. Delaplaine, and T. Cannon; AIAA Conference on Space Life Sciences; September 1994). In short, initial embryological studies require gross morphological visual observations over the entire developmental time course, the ability to control the environment of the embryos in terms of oxygen, media flow, and temperature, and the ability to fix embryos at appropriate times for subsequent sectioning and more detailed analysis.

Thirty-six *medaka* embryos were flown on a modified STL-B hardware system. Embryos were collected within one half hour after fertilization, cleaned of chorionic hairs, and transferred to both flight and ground control STL-B growth chambers. The embryos were held at 11.5-12.0°C until one half hour after reaching microgravity, at which time the temperature was raised to 17.5-18.5°C. Embryos in all chambers were monitored by video which was either broadcast to Earth, stored on videotape, or stored as digitized images. The six chambers of the STL-B were fixed with Bouins fixative at preprogrammed times and the embryos returned to Earth for detailed morphological and molecular analysis. Digital images and real-time video sequences were taken of the flight, and of synchronous ground and non-synchronous ground embryos under identical conditions except for the flight environment (acceleration into orbit and microgravity). Embryos were intended to be fixed at the following intervals: Orbit plus 24, 48, 72, 96, 142, and 166 hours. A hardware malfunction led to the fixation pre-flight of chamber #4 (72 hrs) and the Orbit plus 142 and 166 hour embryos not being fixed until the shuttle landed back on Earth. This was later traced by the STL-B team to intermittent contact on the wiring to the pump board assembly and the flowpath pumps. The wiring was most likely compromised during the exchange prior to NASA turnover. The pumps were checked and functioning properly at the time of turnover. The remaining fixative was injected to the flowpaths during ground processing. The embryos had not reached the hatching phase at this time. Media and gas exchange to the two flowpaths was affected by the intermittent wiring. The effects of the intermittent nature of pumping on the embryos was investigated in the first of three post-flight tests conducted at Bowdoin College (C.R. Phillips).

All of the embryos both flight and control have been embedded and sectioned. No gross morphological abnormalities were observed between the flight and control embryos or between flight or control animals and comparably staged untreated embryos. Analysis of the video and digital data is still under examination. However, some conclusions can be drawn, the most important being that overt development was normal.

Medaka fish embryos are optically clear, allowing direct observation of embryonic development by video-microscopy. Such instrumentation has been developed by our colleagues from Walter Reed as part of the STL-B hardware. The STL-B hardware has flown experiments on the shuttle on three separate occasions. The first, STS 59, was considered a hardware flight test and was not supported by NASA. *Medaka* embryos were flown on this mission and all systems checked out in terms of biocompatibility. The second and third flights, STS 70 and 78, provided the opportunity for a series of video observations and fixation of embryos for subsequent histological examinations on the effects of microgravity on the development of the *medaka* at various stages.

The first video sequence served as a reference for fish development. There is normally a rotation of the animal pole upwards, relative to gravity, and active cytoplasmic rearrangement towards the animal pole following fertilization. Cytoplasmic components are localized to the animal pole as mitosis begins to partition the egg into cells. It is during the early stages of cell division shown here that the dorsal/ventral and right/left axes are determined. All of the major body organs are spatially determined during gastrulation.

The primary on-orbit activities conducted by the crew was to provide the on-orbit reference to the system at 7 hours after launch and to re-program the embryo positions to accommodate any shifting resulting from launch and orbit. This reprogramming optimized the digital image storage and the on-board recordings by centering embryos in the viewfield. Once the embryos were correctly positioned and the video cables set up, it became possible to receive an earlier than planned, additional downlink. This downlink verified embryo viability and positioning. The first planned downlink was operated by Commander Tom Hendricks, who provided us with a much longer than scheduled downlink. During this downlink, each of the six embryo chambers were reviewed and observed. This proved to provide a good baseline for system operation, performance, and status of the embryos. Subsequent downlinks and on-board recordings were conducted automatically by ground commanding of the shuttle systems. In many cases the downlinks were longer than originally scheduled, and on-board recordings were more frequent than scheduled. The landing preparation reference was provided following our final scheduled downlink and the termination of the last samples.

Following the mission, ground and flight digital images were decompressed and recorded onto compact disks. Copies of these disks have been provided to C.R. Phillips for analysis.

Studies of Complete Life Cycle of *Medaka* Embryos Which Were Allowed to Hatch Upon Return to the Earth Environment: Two of the culture chamber containing embryos in the experiments flown in STS 70 were returned to earth unfixated. These embryos and one chamber of control embryos were held at KCS for 9 days at 18°C. They were then shipped to Columbia University in a container to maintain the 18°C temperature. Upon receipt on 7/25/95, they were placed in a controlled temperature environment of 21-25°C, average temperature was 23°C. The lighting regimen in the culture incubator was 12 hours light : 12 hours dark from 7/25/95 to 8/17/95. After 8/25/95, the light/dark cycle was changed 16 hours light : 8 hours dark. The average hatching time, in days post arrival at Columbia University, was 30 days for the flight animals (two groups of six embryos each) and 40 days for the control (one group of 6 embryos). Both series are considerably longer than the typical hatching time for medaka of 10-14 days total at 25°C. This delayed hatching in embryos in the culture chamber is potentially interesting and should be investigated further; however, the numbers in the present study were too small to be evaluated statistically.

Upon hatching, the fry were allowed to develop into adulthood. Over a 4 month-period, 3 control animals and 5 experimental animals survived and began to reproduce. The two females and one male of the control group produced 8 eggs that were recovered but then stopped reproducing, no doubt due to the small number of animals in the tank. In contrast, the space flight animals consistently yielded batches of fertilized embryos from 11-10-95 through 7-16-96. This result demonstrated that animals exposed to microgravity during embryogenesis could reproduce. A sample of the embryos yielded from the flight animals were then removed to a separate culture environment and allowed to develop to adulthood. Upon reaching sexual maturity, these animals began to mate as well. Thus the progeny of the space flight animals are also fertile.

Ground-based studies:

Analysis of Gene Expression in *Medaka* Embryos: We have begun to analyze the expression of the *medaka Hoxa-4* gene as a marker of embryonic development for analyzing the effect of microgravity on pattern formation and embryonic segmentation. To this end, we are currently determining the expression pattern of *medaka Hoxa-4* during embryogenesis, under normal conditions. Our Northern blot analysis of total RNA isolated from embryos pooled at various stages of development revealed the expression of a major transcript of ~1.7kb, first detected at stage 21, when the *medaka* embryos have six to eight somites. Our next experiments will extend this analysis to sections of embryos at various stages of development by *in situ* hybridization on histologically sectioned embryos. This will be critical for studies on the expression of specific genes in flight embryos, as multiple genes could be assayed in the same embryo. We have concomitantly successfully obtained whole mount *in situ* hybridizations with *medaka* embryos, but feel it is critical to develop the use of sectioned material to maximize data return.

Given the growing opportunities for long-duration flights of human beings in space, it is crucial to determine the effects of microgravity stress during space flights on the various aspects of human life. One of the most fundamental aspects is reproduction. We still have little information about the possibility of normal vertebrate reproduction in space. Given the inherent difficulties in mammalian models in space flight investigations, we are undertaking the present studies using an alternative vertebrate model, the fish *Medaka*. The basic hypothesis to be examined is that animal embryonic development, and neural development in particular, would be affected, potentially in a subtle but biologically significant manner, by exposure to the environment of space, and further, that this response may differ at different stages of embryonic and postnatal development of the animal.

It is commonly believed that some species make use of Earth's gravitational field as a positioning cue during early embryogenesis. It is our intention to determine: 1) if embryos can develop normally without such cues; 2) to determine if some stages of early embryogenesis are affected; and 3) at which point the affected stages regulate back to producing normal embryos. Through study of animal development, the ultimate objective is to understand the potential effects on human embryonic development and determine the risks on human

reproduction induced by microgravity stress during long lasting space flights. Our studies address aspects of fundamental biology concerning vertebrate development at the molecular, cellular, and physiological levels in the environment of microgravity. Since the studies are conducted on vertebrates, the results can be more readily extrapolated to other mammalian models, particularly humans. It is commonly accepted that normal development rests largely on the embryo's ability to maintain a highly coordinated program, temporally and spatially, of morphogenetic events. Interference with the normal program of development, that is, an alteration in the carefully orchestrated cell-cell interaction, cellular migration, and cell death that should be occurring during normal embryogenesis, could result in development abnormalities at morphological, physiological, behavioral, and other levels. These abnormalities could be evident immediately or might not be apparent until later in life. Animals that develop, are born, reared, and reproduce in space may exhibit profound or more subtle morphological, physiological, behavioral, and other changes that our experiments should help to evidence. In addition, our studies have the additional long-term potential of providing a vertebrate model for studies on the effects of others aspects of the flight environment, including radiation, as *Medaka* fish has been used as a vertebrate (but non-mammalian) test system for studying radiation-induced mutagenesis.

FY97 Publications, Presentations, and Other Accomplishments:

Wolgemuth, D.J., Herrada, G., Kiss, S., Cannon, T., Forsstrom, C., Pranger, L.A., Weismann, W.P., Pearce, L., Whalon, B., and Phillips, C.R. "Vertebrate development in the environment of space: Models, mechanisms, and use of the *Medaka*." ASGSB Bull. (In Press).

Role of Corticosteroids in Bone Loss during Space Flight

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Funding:

UPN/Project Identification: 106-30-32

Solicitation: AO-OSSA-84

Initial Funding Date: 1993

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 284071

Flight Assignment: LMS (STS-78, June 1996)

Responsible NASA Center: Ames Research Center

Task Description:

Corticosteroids are hormones produced by the cortex of the adrenal gland in response to stress, and their overabundance inhibits the growth of bones and leads to loss of bone mass. In-flight blood samples from astronauts and cosmonauts have revealed increased levels of plasma corticosteroids, particularly cortisol, raising the question of whether the production of excess corticosteroids in response to the stress of orbital flight may contribute to the human bone loss associated with space missions.

Twenty-four male rats will be the subjects in this experiment. Before and after the mission, data will be gathered on their bone mass, levels of bone formation and resorption, and bone cell activity to determine the effects of space flight. Each rat will be injected before the mission with a calcein label that binds to the calcium on bone-forming surfaces. After the mission, scientists can determine how much bone growth has occurred by measuring the amount of bone deposited over the label.

Adrenal glands of laboratory rodents exposed to extended weightlessness have shown evidence of hypertrophy, an increase in size, which results in increased blood levels of corticosteroids. To eliminate the source of corticosteroids, six of the flight rats (three per Animal Enclosure Module (AEM) enclosure) will have had their adrenal glands removed a few days before launch. Then, these rats will be implanted with hormone pellets that will release normal levels of corticosteroids into their systems. The other six flight rats, with adrenal glands, are expected to experience adrenal enlargement during the flight and an increase in corticosteroid output. A control group of 12 rodents, 6 of which also have had adrenalectomies, will live in 2 identical AEMs on the ground while the Life and Microgravity Spacelab (LMS) is in orbit.

After the mission, blood and bone samples from intact and adrenalectomized rodents, both flight and ground populations, will be examined. Blood samples will be assayed for plasma corticosteroids, and bone samples will be studied to identify whether any skeletal abnormalities have developed in the flight rodents in the absence of corticosteroid excess.

All preflight procedures were accomplished as planned. The rats were successfully adrenalectomized (ADX) and the implanted hormone pellets delivered physiologic levels of corticosterone and aldosterone to the systemic circulation. The substantial increase in body weight that occurred in all rats indicated that the ADX-supplemented rats were healthy and that the flight rats tolerated weightlessness well. The observed adrenal hypertrophy in the intact sham flight rats was also a positive finding in that it was suggestive of corticosteroid excess in these animals.

Bone histomorphometry was performed at several skeletal sites. These analyses revealed a lack of bone changes in all groups of rats, including intact flight rats. The latter animals exhibited normal cancellous bone mass at 4 different skeletal sites. Furthermore, both cancellous and cortical bone formation were found to be normal in flight rats compared to ground-based control rats. These results clearly indicate that space flight has minimal effects on bone mass and bone formation in rapidly growing rats. Therefore, the experimental objective, which was to test the hypothesis that corticosteroids contribute to bone loss during space flight, could not be achieved due to lack of bone changes in intact flight rats.

The negative findings of the current study emphasize the importance of rat age, strain, and housing conditions for the development of bone changes during space flight. These factors are crucially important for the planning of future experiments involving use of rats as an animal model for the adverse skeletal effects of space flight.

The proposed research will contribute to a more complete understanding of the cause of bone loss during space flight. Such an understanding is critical for the rational design of therapeutic regimens to prevent bone loss in astronauts. This is an important consideration for maintaining the skeleton of astronauts during long-term human occupancy of the International Space Station.

Expression of Contractile Proteins in Microgravity

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available
 Initial Funding Date: 1995
 Students Funded Under Research: 1
 FY 1997 Funding: \$22,000

Solicitation: 93-OLMSA-06
 Expiration: 1998
 Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9306013
 Flight Assignment: NASA-Mir-1B, SLM-1A
 Responsible NASA Center: Ames Research Center

Task Description:

The regulatory contractile proteins troponin T and I are of fundamental importance in the normal physiological function of cardiac and skeletal muscle. For example, troponin T is essential for calcium-dependent myofibrillar ATPase activity and force development. A carefully orchestrated, developmentally regulated change in the expression of the isoforms of troponin T and troponin I occurs in cardiac and skeletal muscle. The troponin T and troponin I isoforms have physiological and biochemical importance. The isoforms alter these myofibril properties. The mechanisms that control these regulated changes are not yet defined.

The study of the effects of microgravity on troponin T and troponin I isoform expression in the quail is most pertinent to the human. Birds and humans demonstrate similar developmental changes in the isoforms of these regulatory proteins in cardiac and skeletal muscle. Using microgravity as a perturbation to alter the expression of these isoforms has the potential for revealing the systems that alter gene expression and alternate splicing of the primary transcript in cardiac and skeletal muscle in the human on Earth. A microgravity-induced interference in the normal development of troponin T isoform expression could enhance or deleteriously affect the relation between myofibril isoform content and calcium transient. Microgravity-induced changes in expression of the troponin T and I isoforms *in ovo* may mimic the effects of microgravity in the amniotic fluid cushioning milieu *in utero*, making the avian results relevant to human development in space. In human heart disease, cardiac troponin T isoform expression is altered. These changes in expression are correlated with changes in myocardial function as described by myofibrillar ATPase activity. Understanding the mechanisms through which isoform expression is regulated may provide a mechanism through which gene and isoform expression can be altered in the patient with heart disease. Furthermore, an understanding of the basic processes that control cardiac and skeletal muscle can be achieved. This understanding may prove useful in the treatment of human disease.

We have successfully purified RNA from fixed tissue in amounts sufficient for us to perform reverse transcriptase-polymerase chain reaction (RT-PCR) experiments. We obtained two PCR products using primers

based on rabbit cardiac troponin T (cTnT) cDNA. The sequences from an alternatively spliced region and that from a central highly conserved region were of the appropriate size.

To ensure our ability to purify RNA from fixed quail tissue and to test the integrity of the RNA for template stability and for generation of species-appropriate products, we performed RT-PCR using primers based on the quail b-actin gene: Forward 5' CCTTCCTGGGCATGGAGTCCT 3'; Reverse 5' GGAGCAATGATCTTGATCTTC 3'. Our negative controls were negative while our positive controls and our experimental products generated the appropriate RT-PCR product. These results were the same using RNA harvested from fixed or fresh tissue.

We pursued the quantitation of differential expression of the alternatively spliced cTnT region during embryonic development using agarose gels and then SDS-PAGE. The latter was used to provide a more dynamic range for quantitation of the two anticipated products which differ in length by 30 nucleotides. In comparing products from embryonic tissue at different stages of development, no difference in the relative amounts of the two products were found. We then compared these products to those obtained from RT-PCR reactions using RNA from the heart of an adult quail. Again no difference was seen in the relative amounts of the two products. These findings are inconsistent with the expression of the two isoforms at the protein level. We subsequently cloned and sequenced the PCR products and found one to be from contaminating rabbit cTnT cDNA. In contrast, no such contamination was present from the PCR product from the central highly conservative region.

We subsequently generated multiple pairs of cTnT primers and used them in PCR reactions with lower stringency. These experiments generated, as anticipated, multiple PCR products. These were sub-cloned and sequenced. To this date, we have not obtained appropriate cTnT sequences.

To obtain useful primer pairs, we are cloning the two quail cTnT cDNAs using a primer strategy based on regions of the molecule that are highly conserved across phyla. We will reproduce our successful amplification of a central cTnT region and sequence this product. If this general approach is not successful, we will generate a quail heart expression library and obtain from it the necessary sequences to perform our quantitative RT-PCR and examine the effects of microgravity on striated muscle gene expression.

Our modification of previously published methods for obtaining RNA from fixed tissues results in a markedly greater yield of transcript. RT-PCR of these transcripts yields appropriate products when species-specific nucleotide sequences are used to generate the primer pairs. Although fragmentation of RNA by fixation has been described, we are able to obtain RT-PCR products in excess of 200 base pairs using tissues that have been in fixative for over six months.

Individual embryonic hearts from fixed embryos yield sufficient RNA to perform RT-PCR. The resulting products are of adequate length for assessing regulation of gene expression.

This proposal aims to examine in Japanese quail the effect of microgravity on the developmentally programmed expression of troponin T and troponin I isoforms, two sarcomeric thin filament proteins that regulate cardiac and skeletal muscle contraction. A similar developmental profile in the expression of these proteins occurs in the human and the bird. We hypothesize that microgravity will alter the pattern of expression of slow skeletal muscle and cardiac troponin I in cardiac muscle and those of the cardiac and skeletal muscle troponin T isoforms. Given the similarity of the developmental programs in humans and birds, the results of this study will be relevant to human development and disease.

The protocols developed in this investigation will prove useful in studying gene regulation in microgravity. The stability of RNA in fixed tissues allows fixing of solid tissue and blood in microgravity and subsequent analysis many months and potentially years after the tissue was harvested. Thus, gene expression and its modification by microgravity can be examined over time in the adult and during development in the embryo and fetus. Our improved method for harvesting RNA from fixed tissues will enhance the study of archival pathologic specimens. This approach will enable the examination of such tissues for the molecular basis of cancer, genetically inherited syndromes, and congenital defects.

Inflight Radiation Measurements

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Funding:

UPN/Project Identification: 5.2.1

Solicitation: US/RSA Negotiations

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Joint Agency Participation: NASA/RSA

Flight Information:

Experiment ID: 9400521

Flight Assignment: SLM-1A (Spacelab-Mir)

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: TLD Passive Radiation Detector, TEPC

Task Description:

The project was designed to measure radiation dose and dose equivalent rates using both passive (thermoluminescent detector) and active (tissue equivalent proportional counter) at a number of locations on the Mir station during the NASA-Mir program. To date data has been acquired from March 1995 to the present time.

FY97 Publications, Presentations, and Other Accomplishments:

Badhwar, G. Results of radiation dose measurements from the NASA Mir 3 mission. NASA Tech Brief, (1997).

Badhwar, G.D. "Drift rate of the South Atlantic anomaly." J. Geophysical Res., vol. 102, no. A2, 2343-2349 (1997).

Badhwar, G.D. TEPC results from NASA-Mir 4. NASA Tech Brief, (1997).

Badhwar, G.D. and Johnson, A.S. Measurements of radiation dose rates during the NASA-Mir 2 mission. NASA Tech Brief, (1997).

Badhwar, G.D., Shurshakov, V.A., and Tsetlin, V.V. "Solar modulation of dose rate onboard the Mir station." IEE Transactions Nucl. Sci., vol. 44, no. 6, (1997).

Environmental Radiation Measurements on Mir Space Station

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Funding:

UPN/Project Identification: FBI 3 and FBI 4
Initial Funding Date: 1995
Students Funded Under Research: 4
FY 1997 Funding: \$150,000

Solicitation: 94-OLMSA-01
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9401620
Flight Assignment: NASA-Mir-1B
Responsible NASA Center: Ames Research Center
Flight Hardware Required: Area Passive Dosimeters, External Dosimeter Array

Task Description:

Exposure of crew, equipment, and experiments to environmental radiation during extended space missions such as space station habitation and planetary exploration poses complex scientific and technological problems which need to be resolved before accurate prediction of accumulated doses and adequate radiation protection can be achieved. The development of environmental cosmic ray and trapped radiation models and of computer codes for propagation of radiation through matter is essential to the space radiation protection effort, so that dose rates in spacecraft can be predicted from orbit, date and duration of flight, and the physical attributes of the spacecraft. Detailed experimental mapping of the space radiation environment is necessary for comparisons with and rectification of the predictive models and codes. The NASA-Mir Program provides an opportunity to extend the present database of U.S. measurements of the space radiation environment to the 51.6 degree inclination of the Mir space station orbit. Since the U.S./International space station is likely to occupy a similar orbit, radiation measurements on Mir can also be used for extrapolation of dose rates to the U.S. space station environment. Intercomparisons of U.S. and Russian space radiation measurements from both passive and active detectors are needed to determine the equivalence between different instruments and techniques. Project 1-Internal is a three-year program to perform a systematic series of passive radiation-detector exposures on Mir. Concurrent measurements of absorbed dose, LET spectra (LET >5 keV/micron in water) will be made inside Mir. In Project 2-External, depth dependence of absorbed dose and LET spectra will be measured under thin shielding with dosimeter stacks on the external surface of the Mir. The internal measurements will be compared with measurements from Russian dosimeters and the JSC-TEPC active microdosimeter, exposed in the same location, and all measurements will be compared with calculations made for similar conditions by the currently available space environment and radiation transport models. The combination of internal and external measurements will yield detailed information on shielding effectiveness in the 51.6 degree orbit. The systematic series of

measurements made during the approach of solar minimum (September 1997) will measure solar cycle effects on environmental radiation levels and include the maximum doses of galactic cosmic rays for this cycle.

Area Passive Dosimeters (APDs) from the NASA-2/Mir-21 and NASA-3/Mir-22 mission were returned to University of San Francisco and detector readout and analysis is underway. The External Dosimeter Array was deployed on 29 April 1997.

Other activities of this research include:

- Measure mission dose equivalent rates and LET spectra using passive dosimeters on NASA-2 and -3.
- Map internal radiation environment of Mir using APDs located in different Mir modules (Core and Kvant-2).
- Determine radiation environment external to Mir with measurements of depth dependence of dose and LET spectra on the outer surface of Mir.
- Measure shielding effects of Mir using combined internal and external dosimeters.
- Intercompare dose equivalents and LET spectra measured by active (JSC-TEPC) and passive (PTNDs, TLDs) dosimeters.
- Intercompare U.S. and Russian dosimeters.
- Compare experimental and calculated dose equivalents and LET spectra for rectification of environmental models of trapped and GCR particle spectra and of codes used for propagation of radiation through matter.

FY97 Publications, Presentations, and Other Accomplishments:

Benton, E.R., Benton, E.V., and Frank, A.L. "Preliminary results from the environmental radiation measurements experiment aboard the Mir Station." 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, Long Island, NY (April 29 - May 3, 1997).

Benton, E.V. and Benton, E.R. "Environmental radiation measurements experiment on Mir Station." Phase 1 Research Program Results Symposium, Johnson Space Center, Houston, TX (August 5 - 7, 1997).

Shinn, J.L., Wilson, J.W., Benton, E.V., and Badavi, F.F. Multilayer analysis of an iron radiation beam experiment. NASA Tech Brief, NASA TM-4753 (1997).

Adaptive Changes in Cardiovascular Control at μ G

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Funding:

UPN/Project Identification: E712	Solicitation: 94-OLMSA-01
Initial Funding Date: 1995	Expiration: 1999
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$441,000	

Flight Information:

Experiment ID: 9401712
Flight Assignment: NASA-6/Mir 25; NASA-7/Mir 26
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: Chibis, Transcranial Doppler, CBPD, GASMAP, ECG System, etc.

Task Description:

The present studies share a common approach with our experiment "Integration of Neural Cardiovascular Control in Space," scheduled for Neurolab (1998), including collaboration with Doctors Baich et al. (DLR), Eckberg et al. (Virginia Commonwealth University), and Robertson et al. (Vanderbilt University).

The broad objective of this experiment is to explore and define the mechanisms by which the autonomic nervous system regulates the circulation to support tissue perfusion, particularly in the brain, during adaptation to microgravity and readaptation to 1-G. The primary hypothesis is that adaptation to the unique environment of microgravity minimizes the dynamic demands on the cardiovascular neural control. The level of physical activity is decreased, and no postural adjustments are required. This regulatory environment is likely to degrade important neurohumoral control mechanisms.

The experimental design represents an integrated approach to the testing of this primary hypothesis. The following questions will be answered: 1) Does efferent sympathetic nerve activity increase appropriately in response to baroreflex and non-baroreflex-mediated stimuli after space flight? 2) Can integrated clinical tests of autonomic function detect functional impairment and can they be used to characterize the time course of adaptation to microgravity? 3) Does regulation of the cerebral circulation change in parallel with or independent of the regulation of the systemic circulation? 4) Can advanced mathematical models of neural control including both linear and non-linear dynamics be developed to gain insight into the integration among neurocirculatory

variables and control mechanisms? A series of well-defined physiological stimuli has been defined, including lower body negative pressure, a cold pressor test, isometric exercise, Valsalva, and controlled breathing. Responses are characterized by multiple measurements including heart rate, continuous finger arterial pressure and direct recording of muscle sympathetic nerve traffic. The U.S. Mir experiments will enable us to extend the Neurolab observations to flights of long duration.

An experiment including many components of E712 was implemented on the German Mir '97 (PI - F. Baisch, DLR) with the E712 team as Co-Is.

Members of the experiment team participated in preflight and postflight data collections for Mir '97 in Cologne and Star City. Data analysis is in progress.

The experiment was initially assigned to U.S. Mir Phase 1B (NASA 6/Mir 24). Crew training and baseline data collection activities were accomplished during May and June 1997. The experiment was then transferred to Phase 1B (NASA 7/Mir 25). Crew training and baseline data collections were performed at JSC during September 1997.

The experiment will provide new data on human cardiovascular control mechanisms. Orthostatic hypotension is a common and important condition in astronauts early after return from space and is also a common clinical problem. The experiment is likely to provide new and specific information on pathophysiological mechanisms which is highly relevant to both general clinical practice and to flight medicine. The experiment is also likely to contribute to the development of new approaches to the diagnosis and functional evaluation of patients with orthostatic intolerance.

The Effects of Long-Duration Space Flight on Eye, Head & Trunk Coordination During Locomotion

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Funding:

UPN/Project Identification: not available	Solicitation: 94-OLMSA-01
Initial Funding Date: not available	Expiration: not available
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$325,000	

Flight Information:

Experiment ID: 9400422
 Flight Assignment: NASA-Mir-1B
 Responsible NASA Center: Johnson Space Center

Task Description:

In the microgravity environment of space flight, the relationship between sensory input and motor output is altered. During prolonged missions, neural adaptive processes come into play recalibrating the central nervous system (CNS) to permit new sensory-motor strategies to emerge in the novel sensory environment of microgravity. However, the adaptive state achieved on orbit is inappropriate for a 1-G environment leading to postural and gait instabilities and disorienting illusions of self and surround motion during head movement on return to Earth.

Sensory inputs from the vestibular, proprioceptive, visual, and deep-pressure systems are used to modify the basic central nervous system scheme to produce appropriate gait patterns for each situation. Interlimb coordination and movement of the head-trunk ensemble require integrated muscle activity patterns of relaxation and contraction of the leg. Current investigations clearly demonstrate that during walking and running, the head is stabilized with respect to the Earth's vertical in a very precise fashion. This suggests that postural and gait motor control strategies are organized around achieving the goal of head stabilization thus ensuring gaze stability and the maintenance of visual acuity during locomotion. Extended exposure to microgravity may exacerbate gait, head, and gaze instabilities during readaptation to a 1-G environment, resulting in slower acquisition of terrestrial locomotor strategies.

The general objectives of the proposed research are to (1) characterize pre- and postflight eye-head-trunk coordination during treadmill locomotion, and (2) define the pre- and postflight energy transfer between the lower limbs and the head, lower limb kinematics, and muscle activation patterns during overground locomotion.

To accomplish these objectives, crew members will perform two separate locomotion tasks: (1) walking and running on a motorized treadmill, and (2) unrestrained overground locomotion. During the treadmill locomotion task, targets will be placed at different distances from the subject for visual fixation. A video-based motion-analyzing system and accelerometers will be used to measure head and body movement while standard DC-electrooculographic recording methods will be used to measure eye movements. Muscle activation patterns will be determined by recording electromyographic (EMG) signals from the muscles of the leg and postural muscles of the neck and back.

Summary of progress: (1) Integration of video motion analysis system with force plate, accelerometer, electromyographic (EMG) and EOG data acquisition systems (see below for details). (2) Set up and test of hardware in Star City, Russia. (3) Mir-21 pre and postflight data were collected on two subjects. Three preflight (120, 45, and 10 days before launch) and four postflight (1, 3, 7, and 180 days after landing) data collections were performed. One preflight session was collected at the Johnson Space Center (JSC). All other data collections occurred at the Gagarin Cosmonaut Training Center (GCTC), Star City Russia. (4) NASA-3 pre- and postflight data were collected on one subject. Three preflight (120, 45 and 10 days before launch) and five postflight (4 hours, 1, 5, 7, and 12 days after landing) data collections were performed. One preflight data session was collected at GCTC; all other data collections occurred at JSC. (5) Mir-22 pre- and postflight data were collected on two subjects. Three preflight (120, 45 and 10 days before launch) and four postflight (1, 3, 7, and 11 days after landing) data collections were performed. One preflight session was collected at JSC. All other data collections occurred at the GCTC. (6) NASA-4 pre- and postflight data were collected on one subject. Three preflight (120, 45, and 10 days before launch) were collected at JSC, and four postflight data collections (4 hours and 1 day after landing collected at Kennedy Space Center (KSC) and 6, 9, and 13 days after landing (collected at JSC) were performed.

Hardware Integration Summary: Significant locomotor and postural equilibrium disturbances frequently occur after space flight. Previous investigations have typically assessed how specific sensory-motor sub-systems adapt to weightlessness and return to 1-G. While this approach has yielded significant gains in our understanding the adaptation process, the development of an integrated data acquisition system will allow for the investigation of the interaction and synergies of various sub-systems used to produce coordinated movement strategies during locomotion. Simultaneous collection of the many variables necessary to perform comprehensive investigation of these locomotor strategies after space flight involves the integration of multiple data acquisition systems. We have developed a data acquisition strategy which allows us to obtain continuous measurements of various kinematic, kinetic and physiological variables, during protocols involving overground and treadmill locomotion during visual target acquisition. We are implementing this strategy with Experiment 644.

During the locomotion protocols, the following data are collected: (1) three dimensional full-body segmental kinematics using video motion analysis; (2) triaxial shank and head accelerations; (3) surface EMG from the neck, trunk, and right lower limb; (4) vertical and horizontal eye movements using DC-EOG; (5) heel strike and toe off using footswitches; (6) ground reaction forces during overground locomotion; and (7) dynamic visual acuity measures during treadmill walking. The following equipment is integrated to form the data acquisition system: (1) a six camera, high resolution video motion system (Motion Analysis Corp., Santa Rosa, California); (2) two triaxial accelerometers (Entran Sensors and Electronics, Fairfield, New Jersey); (3) a seven channel pre-amplified surface EMG amplifier system (Therapeutics Unlimited, Davenport Iowa); and (4) a motor-driven treadmill (Quinton Instrument Co., Seattle, Washington). The data are simultaneously collected using commercially available data acquisition software and A/D boards on two PCs and a Sun Workstation. The onset of data collection is synchronized with the use of a sync pulse generated by the Motion Analysis acquisition software. Since experimental objectives mandated that high-resolution, full-body kinematics be collected during both overground and treadmill locomotion in a short postflight testing period, it was necessary to minimize transition time between the two protocols. This was accomplished by precise positioning of the

six cameras such that the resolution was maximized in both configurations and minimal camera movement was required to reconfigure. This set-up was successfully implemented and used to collect baseline data on subjects in Star City, Russia.

This investigation is one component of an integrated program of Neuroscience experiments being conducted at Johnson Space Center designed to examine microgravity-induced adaptive modification of spatial orientation and motion perception processes, gaze control mechanisms, and postural and locomotor control. These investigations are aimed at determining the magnitude and time constants of adaptation to microgravity and readaptation to Earth gravity as a function of space flight mission duration.

Performing this investigation following extended stays on the Mir (90 and 180 days) will serve to significantly supplement our present short-term shuttle data set. Importantly, it will provide a measure of long-term adaptive changes in locomotor control that will help us further understand and interpret the results obtained following relatively short microgravity exposures on shuttle flights.

In addition to addressing crew health and safety, this research will also further our understanding of clinical gait syndromes. NASA and the National Institute of Aging (NIA) have recently entered into a collaborative agreement to pursue research topics of common interest. Both the ages population and returning space travelers experience postural and gait instabilities. However, in the case of returning astronauts, observed adaptive changes are truly plastic as they resolve themselves following interaction with the terrestrial 1-G environment (at least for flights up to 14 days in duration). Alternatively, in the aged population, postural and gait instabilities may persist surpassing the ability of the CNS to adapt and compensate for dysfunction. However as we investigate adaptive changes associated with flight of longer duration, we may find changes are not so fully reversible. Understanding how the CNS adapts to change and exploring the limits and range of plastic modification, whether it is aging or lack of a gravity vector, is central to the NASA/NIA collaborative effort.

The development of unique research protocols like the ones that have been developed in this study can be used by clinicians to evaluate rehabilitation techniques for patients with balance and gait disorders. Development of this new technology can lead to the establishment of worldwide clinical vestibular testing norms that can be used in medical facilities. In addition, this research can lead to the formulation of new models of neural activity based on known pathways and substrates. These models can be used to make predictions about response properties and transfer effects of a variety of motor subsystems following exposure to microgravity or as a predictive tool in clinical conditions.

FY97 Publications, Presentations, and Other Accomplishments:

Bloomberg, J.J., Layne, C.S., McDonald, P.V., Smith, S.L., Mulavara, A.P., Lange, G.W., Pruett, C.J., Merkle, L.A., Riley, P.O., and Kozlovskaya, I.B. "Effect of space flight on head stability during locomotion." Neural Control of Movement Symposium, Cozumel, Mexico (April, 1997).

Bloomberg, J.J., Mulavara, A.P., Riley, P.O., Layne, C.S., McDonald, P.V., Smith, S.L., and Kozlovskaya, I.B. "Three dimensional head movement control during locomotion after long-duration space flight." 12th Man in Space Symposium, Washington, DC (June, 1997).

Bloomberg, J.J., Peters, B.T., Huebner, W.P., Smith, S.L., and Reschke, M.F. "Locomotor head-trunk coordination strategies following space flight." *J. Vestib. Res.*, 7, 161-177 (1997).

Hillman, E.J., Bloomberg, J.J., McDonald, P.V., and Cohen, H.S. "Dynamic visual acuity while walking: A measure of oscillopsia." *J. Vestib. Res.* (In Press).

Hillman, E.J., Bloomberg, J.J., McDonald, P.V., and Cohen, H.S. "Measurement of oscillopsia while walking." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Lange, G.W., Layne, C.S., Pruett, C.J., Merkle, L.A., and Bloomberg, J.J. "The effect of visual target manipulation on neuromuscular patterns during gait." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Layne, C.S., Lange, G.W., Pruett, C.J., McDonald, P.V., Merkle, L.A., Mulavara, A.P., Smith, S.L., Kozlovskaya, I.B., and Bloomberg, J.J. "Adaptation of neuromuscular activation patterns during treadmill walking after long-duration space flight." 12th Man in Space Symposium, Washington, DC (June, 1997).

Layne, C.S., McDonald, P.V., and Bloomberg, J.J. "Neuromuscular activation patterns during locomotion after space flight." *Exp. Brain Res.*, 113, 104-116 (1997).

McDonald, P.V., Bloomberg, J.J., and Layne, C.S. "A review of adaptive change in musculoskeletal impedance during space flight and associated implications for postflight head movement control." *J. Vestib. Res.*, 7, 239-250 (1997).

McDonald, P.V., Layne, C.S., Bloomberg, J.J., and Kozlovskaya, I.B. "Human body shock wave transmission properties after long duration space flight." 12th Man in Space Symposium, Washington, DC (June, 1997).

Merkle, L.A., Layne, C.S., and Bloomberg, J.J. "The use of factor analysis to identify neuromuscular synergies during locomotion." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Mulavara, A.P., Verstaete, M.C., Layne, C.S., McDonald, P.V., and Bloomberg, J.J. "Quantifying coordination in the head-trunk system using a stiffness control paradigm in investigations of adaptations to weightlessness." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Newman, D.J., Jackson, D.K., and Bloomberg, J.J. "Altered astronaut lower-limb and mass center kinematics in downward jumping following space flight." *Exp. Brain Res.* (In Press).

Pruett, C.J., Layne, C.S., Lange, G.W., Merkle, L.A., and Bloomberg, J.J. "The effect of cadence and space flight on neuromuscular patterns during overground locomotion." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Smith, S.L., Layne, C.S., and Bloomberg, J.J. "The effects of space flight on segmental coordination during combined treadmill locomotion and visual target fixation." Houston Conference on Biomedical Engineering, Houston, TX (February, 1997).

Greenhouse (III): Gas-Exchange and Seed-to-Seed Experiments on the Russian Space Station MIR

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Igor B. Podolsky; Institute of Biomedical Problems, Russia
Lola Chernova; Institute of Biomedical Problems, Russia
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Funding:

UPN/Project Identification: 7.1.2
Initial Funding Date: 1993
Students Funded Under Research: 6
FY 1997 Funding: \$300,000

Solicitation: AO-OSSA-84
Expiration: 1997
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 284093
Flight Assignment: NASA-Mir-1B
Responsible NASA Center: Ames Research Center

Task Description:

The Mir Space Station provides an outstanding opportunity to study long-term plant responses to microgravity. Furthermore, if plants can be grown to maturity in microgravity, they might be used in future life-support systems. The primary objective of the Greenhouse experiment was to grow a Super-Dwarf wheat through a complete life cycle in microgravity; i.e., from seed to seed. Additional objectives were to study chemical, biochemical, and structural changes in plant tissues as well as photosynthesis, respiration, and transpiration (evaporation of water from plants). Another major objective was to evaluate the suitability of the facilities on Mir for advanced research with plants. The Greenhouse experiment was conducted in the Russian/Bulgarian-developed plant growth chamber, the Svet, to which the U.S. has added an instrumentation system to monitor changes in CO₂ and water vapor caused by the plants (with four infra-red gas analyzers monitoring air entering and leaving two small plastic chambers). In addition, the U.S. instrumentation also monitors O₂; air, leaf (IR), and substrate temperatures; cabin pressure; photon flux; and substrate moisture (18 probes in the root module). Facility modifications were first performed during the summer of 1995 during Mir 19, which began after STS-72 left Mir. Plant development was monitored by daily observations and some photographs. Plant samples were collected five times during the 1995 experiment for chemical fixation or

drying, and at final harvest. Samples were returned on STS-74 in November 1995. Because four of six light sets failed at the beginning of the experiment, plants grew very poorly; no seed heads were formed. The experiment was repeated in 1996 as part of NASA 3, using a new lamp bank and other equipment. Samples were returned on STS-81 in January 1997. The plants grew extremely well, producing far more biomass than in any other plant experiment in space, but seeds failed to form in the ca. 280 heads that developed. At present, it appears that failure of seed formation was caused by ethylene, a gaseous plant hormone, in the Mir cabin atmosphere.

Green plants from a second planting were harvested when they were 42 days old and frozen in the GN2 freezer.

In 1996, we experienced 100% sterility in our Super-Dwarf wheat grown onboard the Mir Orbital Station. Scanning electron microscopic examination of Mir- and greenhouse-grown wheat florets indicated that florets produced in the microgravity of space developed similarly during the vegetative stage. However, prior to and at the onset of anthesis, florets onboard Mir ceased development. The anthers did not dehisce, resulting in 100% sterility.

Early examination with light microscopy indicated perhaps only one nucleus per pollen grain, whereas normally, wheat pollen is trinucleate at pollination time. Because it is difficult to localize all of the pollen grains in the same field with a light microscope, we approached this problem with a laser confocal scanning microscope. To achieve visibility of the pollen grains with the confocal microscope, we tested several specific fluorescent stains to enhance the location of nuclei in pollen grains in both Mir- and greenhouse-grown Super-Dwarf wheat. After testing several fluorescent stains, we have had success with propidium iodide, which is specific for nucleic acids. Preliminary data are promising.

We are examining Mir- and greenhouse-grown Super-Dwarf wheat ovaries and anthers with a transmission electron microscope. Our preliminary observations have not yet yielded any differences in organelles at the cellular level. In addition to a more detailed examination of the embryo sac and the pollen grains, we are experimenting with cytochemical stains.

Plant physiologists have studied plant responses to gravity for well over a century, but we still have little understanding of how a plant can respond to even slight changes in the direction of gravitational acceleration. Tip a vertical stem of a seedling a few degrees from the vertical, and it will be vertical again within a few hours. Thus it would not be surprising if plants grew abnormally in microgravity. Our experiment suggests that healthy plants can be grown in microgravity, and even that orientation will not be a serious problem. It is highly likely that suitable atmospheric control (e.g., elimination of ethylene) will permit seed formation and development in microgravity. Thus, there is little reason to doubt that wheat and other plants can be used as a food source for future astronauts, purifying the atmosphere in the process. The basic understanding gained in our space experiments, plus the ground studies that support the space experiment, may well have future application in agriculture as well as basic biology.

FY97 Publications, Presentations, and Other Accomplishments:

Biesinger, H., Strickland, D.T., Campbell, W.F., Salisbury, F.B., and McManus, W.R. "SEM and EDX of crystals found on leaves of wheat grown under microgravity conditions." PD-AAAS/WR-ASHS Ann. Meet., Oregon State University, Corvallis, OR (Second Place Award for Excellence in Undergraduate Student Poster Paper Competition in the ASHS-WR) (June 22 - 26, 1997).

Bingham, G.E., Campbell, W.F., Salisbury, F.B., Carman, J.G., Jahns, G., Pletcher, D., Bubenheim, D.B., Yenbdlar, B., Sytchev, V., Levinskikh, M.A., Podolski, I., Ivanov, T., Kostov, P., and Supernova, S. "Integrated plant experiments on the Mir Space Station." Phase I Research Program Interim Results Symposium, JSC, Houston, TX (August 5 - 7, 1997).

Campbell, W.F. "Morphometric and morphological characteristics of wheat grown onboard Mir, NASA-3, Mir-10-20." Science Working Group Conference, Moscow, Russia (July 7 - 11, 1997).

Campbell, W.F., Bubenheim, D.L., Salisbury, F.B., Bingham, G.E., Gillespie, L.S., Carman, J.G., Fljina, G., Tatijana, V., Strickland, D.T., McManus, W.R., Levinskikh, M., Sytchev, V.N., Ivanova, I., Chernova, L., and Podolsky, I. "Morphometric plant growth and light and scanning electron microscopic assessment of reproductive structures of wheat grown on Mir." First Phase 1 Research Program Results Symposium. NASA/JSC, Houston, TX (August 5-7, 1997).

Campbell, W.F., Strickland, D.T., Salisbury, F.B., and Bingham, G.E. "Ultrastructural assessment of reproductive structures of wheat grown on Mir." 13th Annual Meeting of the American Society for Gravitational and Space Biology, Washington, DC (1997).

Jiang, L. Development of super-dwarf wheat under stress conditions simulating those on the Space Station Mir. (Thesis) Utah State University Library (1997).

Jiang, L., Salisbury, F.B., Campbell, W.F., Carman, J.G., and Nan, R. "Studies on flower initiation of super-dwarf wheat (*Triticum aestivum* L.) under stress conditions simulating those on the space station Mir." J. Pl. Physiol. (In Press).

Nan, R. Effects of CO₂, light, photoperiod, and water potential on levels of hormones in wheat (*Triticum aestivum* L.). (Dissertation) Utah State University Library (1997).

Salisbury, F.B., Bingham, G.E., Campbell, W.F., Carman, J.G., and Bubenheim, D.L. "Current status of gas-exchange and seed-to-seed experiments on "Greenhouse-2" during SLM-1. NASA-3, Mir-10-20." Science Working Group Conference, Moscow, Russia (July 7 - 11, 1997).

Salisbury, F.B., Gillespie, L.S., Campbell, W.F., and Hole, P. "Ground-based studies with super-dwarf wheat in preparation for space flight." J. Plant Physiol. (In Press).

Strickland, D.T., Campbell, W.F., Biesinger, H., Salisbury, F.B., and McManus, W.R. "SEM investigations of reproductive morphology of MIR grown wheat." PD-AAAS/WR-ASHS Ann. Meet., Oregon State University, Corvallis, OR (Won First Place Award for Excellence in Student Paper Competition in the WR-ASHS and The AAAS-Wide Robert I. Larus Travel Award) (June 22 - 26, 1997).

Strickland, D.T., Campbell, W.F., Salisbury, F.B., and Bingham, G.E. "Morphological assessment of reproductive structures of wheat grown on Mir." 13th Annual Meeting of the American Society for Gravitational and Space Biology, Washington, DC (1997).

*Effects of Microgravity on Quail Eye Development***Principal Investigator:**

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available
 Initial Funding Date: 1995
 Students Funded Under Research: 1
 FY 1997 Funding: \$15,000

Solicitation: 93-OLMSA-06
 Expiration: 1998
 Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9306010
 Flight Assignment: Euro-Mir
 Responsible NASA Center: Ames Research Center

Task Description:

We proposed that embryonic eye development will occur normally in microgravity. Our studies of chicken embryo eyes from STS-47 (flight vs. controls) have indicated no major differences, except that in the cornea, a much higher than normal percentage of the central cornea area contains cellular processes (pseudopodia, filopodia, long blebs, or neurites) in the extracellular matrix of Bowman's Layer. These inclusions were not seen in corneas of Day 14 or Day 17 chick embryos but are prominent in the corneas of hatchlings that came from embryos that flew in space. To confirm these results from chickens by making corresponding observations in quail, we proposed to examine the eyes of quail embryos that flew in space (and corresponding ground controls). The goal of this series of NASA/Mir investigations was to examine eyes and corneas from embryonic Day 16 (E16) quail for aberrations due to microgravity. We observed nerve development patterns in the corneas, examined corneas for clarity, and observed physical parameters of eyeballs, corneas, and the ossicle (bone) rings surrounding the corneas.

Procedures: Eye Dissection

We removed and studied a total of five eyes from E16 Flight birds (including the right and left eyes from one bird that was fixed outside its shell), 13 E16 Laboratory Control eyes and 9 E16 Synchronous Control eyes. Eyes were removed from those three groups of embryos at the October 1996 dissection at NASA-Ames. Additional postflight control quail were incubated in October 1996 and March 1997, and were dissected in June 1997 dissection. Those post-flight control groups are identified as Synchronous Controls-2 and -3, and Lab Controls-2, -3, and -3-Hi (for high temperature). From those post-flight embryos, we recovered the following numbers of eyes: 8 Synchronous Control-2, 16 Synchronous Control-3, 12 Lab Control-2, 12 Lab Control-3, and 14 Lab Control-3-Hi.

All eyes since the time of recovery have been stored at room temperature in 4% paraformaldehyde fixative.

Procedures: Eye and Cornea Physical Measurements and Observations

We took physical measurements on all eyes, including eye weights, and eye and cornea diameters. Prior to additional procedures, eyes from the October dissection were assigned randomized numbers. Five eyes were selected randomly from each of the treatment groups (Flight, Synch. and Lab) of the October dissection, and the investigators had no knowledge of the group to which each eye belonged. The flight, original controls, and post-flight controls were examined for eye weights, and eye and cornea diameters. Corneal clarity was observed, ossicle rings were stained and observed, electron microscopy was performed on corneas. Corneal transparency was documented by photographing a fine wire mesh viewed through the corneas. Corneas were removed, and the scleral ossicle ring was stained to observe bone numbers and orientation. A wedge-shaped section was removed from the ventral region of each cornea and prepared for transmission electron microscopy.

Procedures: Corneal Nerve Staining

To observe nerve growth patterns, corneal tissue was stained immunohistochemically with antibodies against neurofilaments to detect corneal nerve patterns using a whole-mount procedure just devised in our lab (Barrett et al., in preparation). Numbers identifying the flight and original two sets of controls were decoded following experimental procedures and measurements.

Procedures: Embryonic Day 14 Embryo Dissection and Observations

At the dissection site in California in October 1996, we also made observations on a total of 19 eyes from E14 Flight, Synchronous Control and Lab Control embryos (all incubated on Mir or on the ground in March and April, 1996). Those measurements included eye weight, eye diameter (dorsal/ventral and nasal/temporal), and corneal diameter (dorsal/ventral and nasal/temporal). Statistical comparisons of data from each of the three groups of E14 eyes were made using a completely randomized one-way Analysis of Variance Procedure.

Procedures: Statistical Analyses

A completely randomized experimental design was used. Data were analyzed with a one-way Analysis of Variance (ANOVA) using GB-STAT Statistical Program version 5.4.1 for Macintosh by Richard Taylor, 1995, Dynamic Microsystems, Inc., Silver Spring, Maryland; (originally developed by Philip Friedman, Howard University). Additional statistics were performed using Dunnett's Procedure one-way analysis as necessary.

Findings:

We found no significant differences in means of physical parameter measurements when we compared the eyes of E16 Flight embryos with those from the original Synchronous and Laboratory Controls (both control groups were incubated at the time of the Mir flight, and all three groups were derived from the same batch of fertilized eggs).

When we examined an additional five control groups of E16 embryos (incubated post-flight on the ground from a different batch of eggs than the original Flight and control embryos), we did observe some statistically significant differences. Cornea diameters from quail incubated in microgravity differed from those of these latter control birds. In addition, corneal diameter differences existed between these latter control groups. Corneal clarity observations indicated no consistent differences between Flight embryo tissues when compared with either of the original control groups of corneas. We have not yet determined if differences exist in the ultrastructure of each of the groups of corneas.

We conclude overall that there were probably no consistent significant differences between the eyes from embryos incubated in microgravity and eyes from the control embryos. An important finding from these experiments was that the cornea tissues we examined by electron microscopy from the first three sample groups (Flight, Synchronous Controls and Lab Controls) were poorly fixed, as judged by the presence of empty vacuoles and tissue breakdown. We conclude that investigation should be conducted to improve the method of fixation for further space flight experiments if embryos must be fixed while still within their shells.

The eye is dynamic in at least two respects. First, the eyeball itself develops during embryogenesis much like a balloon or an automobile tire inner tube in that it inflates/enlarges under pressure from within itself. Second,

again like a balloon or a tire, it maintains its structure during adulthood by continuous maintenance of pressure within itself; it does not just inflate itself once and simply become rigid. The fact that the cornea of the eye bulges outward more acutely than the curvature of the rest of the eyeball offers yet another analogy — to that of a defective automobile tire or inner tube in which a weak spot in one wall leads to the formation of an acute bulge. In fact, the cornea develops its differentially acute curvature during embryogenesis specifically because of intraocular pressure and maintains normal structure only so long as that internal pressure is maintained.

When we lean over and put our head between our legs, a lot of fluid shifts from the middle of our body to our head, including the region around the eyes, essentially squeezing the eyeballs from outside, and raising intraocular pressure to an average of 30% above resting levels within 20 sec, rising to levels that significantly overlap those associated with clinical symptoms of glaucoma. When pilots go into microgravity for periods of ~ 20 sec during parabolic flight, a similar shift of fluid occurs from the lower body toward the head and results in an increase in intraocular pressure averaging 50%; this increase occurs each time microgravity is encountered during a linked series of parabolic maneuvers. No data have been published yet about the degree to which the intraocular pressure of astronaut eyes increases in response to entering an environment of microgravity, e.g., on the U.S. Shuttle or on Mir, nor whether the initial, expected increase in that pressure is maintained for long periods of time. If such high intraocular pressures are maintained in astronaut eyes, the chances of developing glaucoma during long missions in space might be substantial. In addition, because the normal embryonic development of the eyeball and cornea depend on the formation of certain levels of intraocular pressures, it will be important to determine whether these structures will be able to develop normally at all in sustained microgravity. If quail are to be used as a renewable food source for long space missions (e.g., Mars), it will be important to determine whether eye and cornea development will be compromised if quail embryos undergo all development in microgravity, as on the Mir station. Our experiments will determine the extent to which the major steps in the embryogenesis of the eyeball in general, and the cornea in particular, can occur normally in microgravity.

On Earth, it is important to learn more about the basic mechanisms of eye development. To be able to study how the developing eye and cornea respond to a new type of physical environment offers a rich array of opportunities for learning more about the eye, those of humans as well as those of quail.

FY97 Publications, Presentations, and Other Accomplishments:

Barrett, J.E. "Eyeballs and quail eggs in space." Slide talks presented to grade school students: Spring Ridge Elementary School sixth grade, Richardson, TX, and Bergman Elementary School, Manhattan, KS (February 24, 1997 and May 8, 1997).

Barrett, J.E., Wells, D.C., and Conrad, G.W. "Increased corneal nerve immunostaining in highly fixed embryonic quail corneas after microwaving, sodium dodecyl sulfate incubation, and hyaluronidase digestion." (Poster) *Investigative Ophthalmology and Visual Science* 38(4):S396 (abstract #1869, Poster #B662)

Skeletal Development in Long Duration Spaceflight

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Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9306003
Flight Assignment: Euro-Mir
Responsible NASA Center: Ames Research Center

Task Description:

The mammalian musculoskeletal system is very sensitive to mechanical loading and weight bearing. We have found in adult male rats that space flight can reduce the rate of new bone formation, alter the muscle-tendon junctional complex, and affect the vasculature within the weight-bearing diaphyseal bone. However, the relative importance of mechanical loading during embryogenesis or during limb development in immature animals is largely unknown. This proposed flight of Mir-1 offers exceptional possibilities because of the long duration of the flight, because tissues will be collected and chemically preserved during the flight, and because enough quail samples will be provided to show statistical significance for any changes which might occur.

Our objectives and methods of study are as follows: (1) To determine the stage of limb development among quail embryos and hatchlings subjected to space flight relative to age-matched controls. This can be achieved grossly by a detailed Faxitron or x-ray analysis of limbs and comparison to controls, as shown for developing rat tibias. (2) To use histochemistry, immunocytochemistry, morphometry, and electron microscopy, as appropriate, to further describe any changes in limb development as a result of space flight. This will provide a more detailed study, at the cellular and tissue level, of any gross changes described in objective 1. (3) To compare development of the long bones, which proceed through a cartilage anlage stage before transforming into bone, to the development of the mandible, which forms bone by a more direct conversion of mesenchymal cells into bone forming cells. This study will also distinguish between space flight effects on cartilage versus bone during the limb development process. (4) To analyze for mineral content of bone and calcifying cartilage using electron microscopy and x-ray microanalysis. This will be augmented with Fourier Transform Infrared microscopy (FT - IR) analysis which will determine any change in mineral crystal size and changes in the organic component of bone.

The long duration of this flight, the comparison of embryonic and hatchling skeletal samples, and the comparison of different cartilage and bone developing systems within the same animal, offer many unique

opportunities for improving our understanding of gravitational effects on musculoskeletal development.

I. Introduction.

Hypothesis:

Previous spaceflight studies have been largely confined to adult animals, in which reduced bone formation and/or mineralization occurred only during the flight period. This flight experiment will study skeletal development during embryogenesis which will permit distinctions between purely genetically driven processes (mesenchymal condensations, cartilage anlage formation) and mechanically or environmentally driven events (bone remodeling, cell replacement, appearance of muscle tissue).

Objectives of the Flight Experiment: (1) Use x-ray (Faxitron) and physical measurements of wing and leg size to determine gross limb and skeletal development. (2) Measure areas of mineralization by image analysis and x-ray microanalysis in limbs and flat bones at different embryonic ages. (3) Use electron microscopy to evaluate mineral deposition within collagen matrix (bone and cartilage). Also combine this data with the distribution of alkaline phosphatase activity, an enzyme necessary for the mineral deposition. (4) Use immunocytochemistry to localize different distribution of collagen types and to describe the organic matrix. (5) Compare the development of flat bones with long bone development. The mechanism of bone formation by intramembranous compared to endochondral mechanisms may be affected differently by space flight.

II. Research Operations.

Preflight:

Preflight activities began 3/15/96 and continued through launch. Inflight activities began at launch and continued at KSC through the first inflight fixation. Remaining inflight activities were monitored from ARC. Ground control activities began 3/27/96 at ARC and continued through final fixation at 4/16/96.

Sufficient numbers of eggs from the hypodynamic strain were not available for this experiment so random-bred *Coturnix coturnix japonica* from Dr. Wentworth's laboratory (University of Wisconsin-Madison) were used. Max/min temperature sensors were included with each egg shipment to determine temperature exposures during shipment. Unacceptable temperatures during transport prevented that particular shipment from being used at KSC.

Upon arrival at KSC, the eggs were processed according to ARC procedure AH-03026. Eggs were weighed, numbered, candled and loaded into the RSKE with an ATR-4 to measure temperatures inside the RSKE. The RSKE was weighed and placed into a preconditioned CRIM and the CRIM monitored until turnover at L-18 hours. Integration was completed on 3/21/96. For ground control purposes, this is considered the start time that the eggs were in the CRIM.

STS-76 launched at 3:15 am on 3/22/96. Contingency and unused eggs were shipped to the University of Wisconsin-Madison for hatching as transport controls. No tissues were taken from transport controls.

Inflight:

Inflight activities were monitored at KSC through day 0 fixation. After returning to ARC the inflight information was received only indirectly. Inflight fixations were performed on 3/25, 3/28, 4/1, 4/4, 4/8, and 4/10/96.

The incubator temperature on 3/27 indicated by voltage readings, was indicated to be 36°C. A medical bulb thermometer placed inside the incubator indicated a temperature of 39°C. Temperature voltage readouts remained relatively constant during the entire incubation. The voltage display corresponded to a temperature of 37-38°C. When the eggs were placed into the incubator, the ATR-4 in the RSKE was also placed into the incubator. This ATR4 was removed from the incubator and returned on STS-79 after 2-3 days in the incubator. This ATR4

indicated that the incubator temperature was close to 40°C. Another ATR4 was removed from the fixation kit and inserted into the onboard incubator; this was to be returned following the termination of the egg incubations.

Ground Controls:

Ground control incubations consisted of a Synchronous control (which normally mimics all flight conditions except weightlessness) and a Laboratory control which is incubated under normal, non-flight conditions. These controls were run in a standard Lyon incubator with automatic rotating of the eggs every hour. The Synchronous control eggs mimicked the flight incubator temperature of 40°C; the Laboratory control incubator was maintained at 37.5°C. These Synchronous controls were not exposed to extra G forces or vibrations similar to flight conditions. Eight eggs were fixed on 4/4, 4/8, 4/11, and 4/15. This was carried out by placing eggs in fixation bags containing 4% paraformaldehyde, then cracking the shell in several places while the eggs was submerged in fixative. Eggs were stored in fixative until dissection on October 7-12, 1996 at ARC.

Additional ground controls were collected in May/June 1997 to control for variations in temperature, vibrational levels, and incubator differences among the control groups (Synchronous and Laboratory Controls). This involved the dissection of 238 embryos which were added to the original study. The original group of Flight, Synchronous and Laboratory embryos will be designated as Group I, in this report. The added controls (238 embryos) will be designated as Group II.

Quality of Data:

The number of embryos collected from this experiment was adequate for scientific evaluation. The dissections were able to provide each investigator with ample numbers of tissue samples. In Group I, the Flight embryos produced 4 embryos out of 12 eggs at D16, 5 out of 8 eggs at D14, 5 out of 8 eggs at D10, and 5 out of 8 eggs at D7. The Synchronous controls provided slightly better yield than the Flight group whereas the Laboratory controls were nearly 100% developed. The lower yield with the Synchronous and Flight eggs might suggest that the higher temperatures (40°C) had a small negative effect on embryonic development.

The survival rate among the Group II embryos was quite high for each developmental period.

III. Results.

Wing: Faxitron (x-ray) images were collected for wing samples from D10, D14, and D16 embryos. These images are being measured for size variation between groups using image analysis techniques on the faxitrons.

Wings were dissected into longitudinal and cross sections and embedded in Spurr's resin. These are being sectioned for light microscopy and image analysis for variation in bone density. Wherever fixation is adequate, samples will also be used for electron microscopy. In any case, analysis of bone mineral for Ca/P ratios will be made as well as analysis of cellular morphology.

Long bones: Faxitron images were made of the intact leg (tibia and femur) to provide bone density estimates and length of calcified portion of each limb relative to cartilage size. The tibia has been embedded in methacrylate and is being sectioned with the mineral intact. From these sections we will analyze Ca/P ratios, relative trabecular bone versus compact bone, cartilage content per bone, growth plate size, and cellular morphology of osteoblasts and osteoclasts. We will use the femur for electron microscopic studies if the tissues are preserved adequately. Preliminary studies with the D14 and D16 mandible suggest that fixation of the long bones may not be optimal for electron microscopy.

Results: Tibial lengths measured at D10, D14 and D16:

The lengths of the tibia were measured from Group I, either directly from the faxitrons (x-ray) by Doty or grossly when the embryo was dissected by Dr. Gurieva. Obviously the gross measurements are larger because they include the overlying skin and muscle whereas the faxitrons only indicate the cartilage and bone.

	FAXITRON (X-RAY)	GROSS (CALIPERS)
E10		
Lab Control	7.0mm (0.8),n=6	8.3mm (0.4),n=7
Synchronous Control	8.2mm (0.6),n=6	9.9mm (1.2),n=6
Flight	7.1mm (0.7),n=4	8.0mm (0.9),n=5
E14		
Lab Control	13.9mm (0.5),n=6	15.4mm (1.1),n=8
Synchronous Control	15.5mm (0.6),n=7	15.8mm (1.6),n=6
Flight	14.8mm (1.6),n=3	16.3mm (1.6),n=3
E16		
Lab Control	16.6mm (0.7),n=5	18.0mm (1.0),n=12
Synchronous Control	16.8mm (0.7),n=6	17.9mm (0.7),n=7
Flight	16.1mm (0.9),n=9	17.0mm (1.4),n=4

Additional Studies with Drs. Gurieva and Dadasheva:

Three samples of legs from E7, E10, E14, and E16 were prepared by these investigators to show the relative amounts of cartilage and bone by Alizarin Red and Alcian Blue staining. We have photographed these samples to measure the amount of cartilage and bone in each. Samples are now being embedded in methacrylate so sections through the bones can be made, and these sections will be analyzed by x-ray microanalysis to determine the relative concentrations of Ca, P, S, and Mg in different regions of each bone. Comparison will also be made between groups. This study is in progress and not yet complete.

Mandibles: Mandibles were embedded in Spurr's resin and sectioned for light microscopy. Some sections were analyzed by x-ray microscopy to produce Ca/P ratios:

Group	Ca:P			
D10 Control	1.33	std. dev.=	0.04	
D10 Flight	1.21	" "	0.05	
D16 Control	1.40	" "	0.02	
D16 Flight	1.40	" "	0.04	

The results indicate normal mineral ratios in the 16D controls and flight samples, but the ratio in the 10D flight was reduced compared to its control. This data is being compared to similar data collected from 10D, 14D, and 16D long bones and wing bones to see if the effect is "real" (not a technical artifact) and if it is a generalized result found in the whole skeleton.

By light microscopy, we measured the relative amount of mineralized bone in the mandibles of the D10 embryos. It appears that there is less mineralized matrix present in the D10 flight compared to the synchronous controls. This would suggest, like the Ca/P data described above, that bone formation at D10 is sensitive to space flight conditions. Infrared (IR) microscopy is being used to analyze the mineral chemistry and the relative collagen:mineral ratios in the sections of mineralized bones. Again, the D10 samples varied from the spectra of the D14 and D16 embryonic mandibles. It appears that the phosphate peak in the D10 flight samples is larger and broader than this peak from the D10 synchronous control. This may be due to the presence of pyrophosphate which can alter the chemistry and solubility of the bone mineral. This finding needs further study and analysis from other D10 bone samples. We also evaluated the mineral:organic matrix content with IR microscopy. This ratio showed no difference between flight and control at D14 and D16. However at D10, the flight sample was significantly different from the D10 control. This difference appears to be due to alteration in the mineral content. Again this finding will be confirmed from analysis of other bone samples (eg. tibia, femur, wing, etc).

Immunocytochemistry:

We have stained some skeletal samples for proteoglycan using special stains and specific antibodies. The only unusual finding to date is the appearance of keratin sulfate in the joint cartilage of the developing long bones. This proteoglycan usually appears during the aging process in older mature animals, so its appearance during development is unclear.

IV Discussion.**(A) Status of Data Analysis:**

Objectives	Group I			Group II
	Flight	Syn	Lab	Lab 2,3,3Hi;Syn2,3
1. Faxitron, physical measurements.	F	F	F	TD
2. x-ray micro-analysis, Ca,P,etc.	F	F	F	TD
3. Electron microscopy.	D7,D10	D7,D10	D7,D10	D7,D10
4. Immunocytochemistry.	PG	PG	PG	TD
5. Comparative development.	TD	TD	TD	D7,D10

Notes: (F) = Finished project. (TD) = to be done.

(D7,D10) = only these two embryonic ages have been completed.

(PG) = only the proteoglycans have been studied, others are in progress.

Analysis of samples for Drs. Gurieva and Dadasheva.

X-ray microanalysis of these samples has been partially completed, but it is apparent that the analysis has to be done in a different way. Therefore we are preparing sections of these calcified bones in order to finish this analysis. This will allow us to analyze specific anatomical areas of the long bones which is what our Russian collaborators had requested.

(B) Final Research Findings:

The final findings must wait for the completed data analysis; however, some results are worth considering:

(1) The faxitron and gross limb measurements showed no significant change in limb size due to development in space.

(2) Even though the long bones which developed in space were the same overall size as control bones, the mineralization at D10 was reduced due to flight. And by D14 and/or D16 this mineralization defect had been corrected back to normal values.

(3) There is some preliminary evidence that the proteoglycan distribution or type of proteoglycan may be altered by flight conditions in the long bones. Since proteoglycans have effects on mineralization, this could be an explanation for the D10 change in mineral content. Thus, when the proteoglycan synthesis or distribution is repaired at D14/D16, then mineralization would also return to normal. We might expect also to find a change in proteoglycan patterns at D7, the earliest time point prior to mineralization at D10, but we have not finished this analysis.

We also need to finish the immunocytochemical study of bone matrix proteins at these different development time periods.

(4) In the higher incubator temperature (L3Hi), we have found the long bones to be advanced in their conversion of cartilage to bone compared to the Lab controls at normal temperatures. Therefore the higher incubator temperatures are not detrimental to bone formation and limb development, and in fact seems to advance the developmental stages.

(C) Conclusions:

The final conclusions must wait until all the data is collected, but some thoughts are already being formulated: Since the size of the limbs is not affected by space flight, then the formation of the cartilage anlage (the first step in endochondral bone formation) and the conversion of mesenchymal cells to osteoblasts (necessary in intramembranous bone formation) are apparently not affected by space flight conditions. However, we did find a mineralization defect at D10 which could be do to: (a) an alteration of the vascular invasion into the cartilage, (b) an alteration in mineral transport from blood to forming bone, or (c) a defect in the matrix undergoing mineralization. We can continue to study the microscopy of the blood vessels and their distribution during development using light and electron microscopy in order to clarify possibility (a). And we will continue the immunocytochemical localization of matrix proteins and proteoglycans to determine the impact of possibility (c). Unfortunately, if the transport of the mineral components was altered due to flight, we will not be able to verify this active transport mechanism, but perhaps some of the vascular and/or ion transport studies of other investigators will help determine the possibility of (b).

(D) Investigation Results:

If our conclusions remain valid during the rest of this study, then we do have a mineralization defect during development which is correctable. However this suggests that some specific stage of mineralization is highly sensitive to microgravity even during development when there is no weight bearing requirement for the skeleton. And since this defect was corrected at later development periods, does this not mean that if we knew what the sensitive stage consisted of, we could find other means to correct this problem? Thus, mineralization defects (i.e., bone loss or reduction) may be correctable for skeletal diseases, not just during developmental periods.

(E) Investigation Applications:

This is partially answered in the paragraph above. We need to find out which step in the mineralization process (which consists of a long complex series of steps) is sensitive to microgravity because it apparently is correctable with further development. The knowledge of this "step" can then be studied and parameters noted in order to use this "step" to try to correct for skeletal diseases and other bone forming problems, such as in osteoporosis.

One of the best documented effects of space flight is the reduction of the musculoskeletal tissues, in mass and mechanical integrity, as a result of non-weight bearing. The loss of bone mass appears to be a problem due to the reduction of new bone formation. This problem will also exist during embryogenesis and is one reason for studying limb development in the quail during space flight. The mechanisms behind this problem may shed new information on human diseases which also suffer from reduced bone formation, such as occurs during aging and in osteoporosis. In addition, the associated muscle and connective tissues which help regulate limb development function in an unknown capacity relative to bone maintenance. By studying these associated tissues during development in space, where there are reduced mechanical forces being applied to the skeleton, we may better understand the normal human physiology and the role played by all these tissues during the aging process or as a result of connective tissue disease.

Autonomic Mechanisms During Prolonged Weightlessness

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Post-Doctoral Associates: 3

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Flight Information:

Experiment ID: 9401709

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

The broad objective of this research is to explore and define the mechanisms by which the autonomic nervous system regulates circulation to support tissue perfusion, particularly in the brain, during adaptation to microgravity and readaptation to the 1-G environment. The proposal for an integrated research program by the Autonomic Control Team has three complementary main goals. First, we will determine, in a definitive way, the adaptive changes in the autonomic nervous system during long-term (about 20 weeks) space flight, and we will utilize this information to obtain insights into various mechanisms that underlie the observed integrated autonomic output. Second, we will determine the adaptive responses (mediated by the autonomic nervous system) through which organ perfusion is maintained during space flight. Third, we will examine the consequences immediately following space flight of any adaptation of the autonomic nervous system that has taken place during space flight, particularly on the various integrated pathways that respond to orthostatic stress in a gravitational field. Tests to be performed include controlled frequency breathing, quantitative Valsalva maneuver, isometric exercise, cold pressor, graded lower body negative pressure, and head-up tilt. We believe that information from these tests can provide insights into the adequacy of afferent input, central processing, and sufficiency of neural and vasomotor responsiveness.

Adaptations that occur at microgravity may physiologically become highly significant after return to the 1-G environment. There are compelling general scientific reasons to take advantage of the access to microgravity to study the dynamic aspects and integration of neural regulation of the cardiovascular system. The unique environment of space with the absence of hydrostatic gradients and the reduction in the overall level of physical activity drastically alters the operating conditions of the circulatory system. Analysis of the effects of

microgravity on specific aspects of neural regulatory mechanisms as proposed in the present study has the potential to produce new information on properties of physiological control mechanisms.

The autonomic function test was successfully performed by the flight engineer of Mir 23. Unfortunately, soon after this initial test, the Mir space station was beset with problems, and we were unable to collect additional data as planned. At the Gagarin Cosmonaut Training Center in Star City, Russia and the Johnson Space Center, we trained NASA astronauts to perform our experiment in space, and collected preliminary baseline data. Soon after training was completed, however, the Progress supply ship collided with the Spektr module, where much of our equipment was stored. Because of the accident, we were unable to study the crew of NASA 6 as planned. We traveled to Star City for postflight data collection on the flight engineer of Mir 23. We now have preflight, inflight, and postflight data on one cosmonaut which we are currently analyzing. New equipment was delivered to the Mir space station with the NASA 6 crew. Although the NASA 7 astronaut has declined to participate in our experiment, we will be studying the Russian crew of Mir 25. We recently collected baseline data on both the Russian commander and flight engineer of Mir 25, and expect that they will be performing our experiment on the station starting in February, 1998. This year our team has suffered a number of setbacks due to the many problems encountered on the Mir space station. Although our potential subject pool has been drastically reduced (from 7 to 3), the data we have collected and will continue to collect will be unique and valuable.

This research will inform issues of great physiological and pathophysiological interest. First, it should improve understanding of a basic physiological mechanism: human cardiovascular autonomic responses to standing upright. Second, it should improve understanding of pathophysiological mechanisms of enormous public health significance. For example, hypertension, which afflicts over 60 million Americans, is associated with impairment of autonomic cardiovascular control. Another example is acute myocardial infarction and a closely related problem, sudden cardiac death. Sudden cardiac death is the largest cause of death in developed countries; the number of people who die suddenly of catastrophic dysrhythmias dwarfs the number of people who die of other public health problems, including AIDS, which attracts much more media attention and research funding. In cardiac patients, abnormal autonomic cardiovascular control (as reflected by impairment of baroreceptor-cardiac reflexes and reduced heart rate variability) indicates which patients are at greatest risk for subsequent cardiac events. Therefore, understanding of how autonomic cardiovascular control mechanisms become impaired may be very important. It is the nature of human research that patients with pathologic conditions are not evaluated *before* they become ill. (Physicians who would study such patients do not know who will become ill.) Therefore, astronauts present a great opportunity. They can be studied before space missions when they are normal, in space as they become abnormal, and after return to Earth as they become normal again. Such longitudinal evaluation of patients is not possible.

FY97 Publications, Presentations, and Other Accomplishments:

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Effect of Microgravity on Afferent Innervation

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No Co-Is Assigned to this Task

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 FY 1997 Funding: \$19,500

Solicitation: 93-OLMSA-06
 Expiration: 1997
 Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9306002
 Flight Assignment: Euro-Mir
 Responsible NASA Center: Ames Research Center
 Flight Hardware Required: Slobak Incubator

Task Description:

The following abstract was submitted and approved for funding with the original application but it was changed due to constraints imposed by hardware, flight schedule, and specimen sharing protocols.

The original proposal was to test the hypothesis that microgravity affects the connectivity of afferent neurons and hair cells in the inner ear and vestibular nuclei neurons in the brain stem of quail (*Coturnix coturnix japonica*) raised in space.

I. One ear of birds available through this NRA will be used to determine, with light and electron microscopy, the branching pattern of afferent terminals contacting hair cells. Specifically we ask: 1) Do chalice, dimorphic and bouton terminals seen in mammalian vestibular organs also exist in birds? 2) Is the ratio of these terminals the same before (E6-E7)1 and after (E10-E12) synaptic genesis in ground controls? 3) Is the ratio of terminals obtained in ground controls altered in birds produced in microgravity? 4) Is the average number of mature synapses the same in ground and control birds?

II. On the opposite ear and brain stem of each animal (ground or flight), the neurofilament protein (NF), the S-100B protein, and the synthesizing and degrading enzymes for the neurotransmitters gamma-amino-butyric acid (GABA) and acetylcholine (ACh) will be demonstrated immunohistochemically. NF will facilitate observing the branching pattern of afferents inside the epithelia, whereas S-100B will show regional variation of ganglion neurons nuclei expressing it in parallel with myelination of axons. A change in the staining pattern of GABA enzymes will reflect changes in the afferent system, whereas a change in ACh enzymes will suggest changes of the efferent system. For light microscopy immunohistochemistry, tissues are embedded in paraffin and cut at 8 - 10 μ m. Each section is saved on a manila folder, and inner ear structures of each embryo are identified.

Sections are then floated in a water bath and affixed to polylysine-coated slides and processed in groups. For electron microscopy of synaptic density of afferent fibers inside epithelia of the equilibrium organs, one ear will be dissected under the microscope after primary fixation with formalin, the utricle-lateral canal ampulla (ULC) separated postfixed and embedded in epoxy. The average number of afferent terminals with structurally mature synapses, in randomly chosen 100 μm^2 areas ($n = 50$) at each age, will be calculated. Knowing if differences in the innervation patterns of inner ear afferents exist between space and ground controls is important because the inner ear contains the organ of balance and equilibrium responsible for motion sickness in space.

Change of original objectives: Aim I of the original application, afferent nerve terminals quantitation with electron microscopy was not done because axoplasmic components of neurons and fibers was extracted during dehydration as result of insufficient cross linking of macromolecules during primary aldehyde fixation in space. On Aim II, GABA and ACh were not demonstrated on space specimens because the preservation of tissue was not sufficient to trap antigens in question. Analysis of ground controls permitted evaluating neuronal proteins and GABA localization, but ACh was not examined.

Reasons for change of original objectives: Many investigators shared specimens from the Mir program. It was necessary to change the original objectives above to accommodate Russian colleagues request for sharing specimens with the American PI. For the present experiment, comparisons between laboratory, synchronous, and laboratory controls were made. Eggs were incubated in a Lyon RX2 incubator at 37.5° C with egg rotation occurring hourly. Synchronous controls were also incubated in a Lyon RX2 incubator with hourly rotation, but the temperature was maintained at 39° to 40° C to simulate the temperature of the Slovakian incubator during space flight. Neither the laboratory nor the synchronous eggs were exposed to acoustics, vibrations, or g load. Embryos were immersed in 4% solution of buffered (pH 7.4) paraformaldehyde at embryonic days 0, 3, 7, 10, 14, and 16. Dr. Fermin received E3, E7, and E10 specimens. Some specimens consisted of one temporal bone and included no brain as opposed to the entire head as it was originally envisioned.

The results obtained thus far suggest that a change in the afferent innervation may take place in embryos exposed to microgravity. In addition, the variables used to simulate flight at 1.0G (synchronous) may retard development of inner ear structures. This observation is consistent with the previous observation of STS-29 chicken embryos that seemed to increase the number of afferent branches inside the basement membrane. To determine if quails, like chickens, also undergo an increase in the number of afferent dendrites after exposure to microgravity, better fixed specimens and modifications of the present experimental conditions should be considered. Additional information about Mir results is found in the web site at <http://www.tmc.tulane.edu/ferminlab>.

What has been accomplished? These preliminary results taken primarily from polychrome, anti-neurofilament and anti-S100 β stained tissues of synchronous and flight E10 embryos suggest that a change in the afferent innervation may take place in embryos exposed to microgravity. In addition, the variables used to simulate flight at 1.0G (synchronous) may retard development of inner ear structures. This observation is consistent with the previous observation of STS-29 chicken embryos that seemed to increase the number of afferent branches inside the basement membrane (Fermin *et al.*, *Histol Histopath.*, 11: 407-426,1996).

What questions have been answered? To determine if quails, like chickens, also undergo an increase in the number of afferent dendrites after exposure to microgravity, better fixed specimens and modifications of the present experimental conditions should be considered. The alternate fixing protocol described should be considered. The increased cross-sectional area of the synchronous control could have resulted from osmolarity changes of the tissues due to saturated fixing solution. Osmolarity alteration in fixative can produce varying artifacts. The difference in the staining pattern of neurons could as well be due to fixation artifacts, but additional samples need to be examined. The effect of fixation on tissue preservation and antigenic site availability for immunohistochemical reaction is an important aspect of anatomical research that has been treated extensively in recent years. While the conditions of ground experiments can not be completely duplicated in flight experiments, we must continue searching for means to improve fixation of live tissues in space.

What new questions have arisen? It is suggested that a double fixation schedule could be designed in a four-bag arrangement to permit replenishing of the fixing solution after a certain time in the space station. Preservation of inner ear tissues was just good enough to allow cytoskeletal components like the neurofilament to remain in the tissues, but there was evidence of cytosol coagulation, vacuolarization, and membrane breaks in all three groups examined and most apparent at E10. E3 and E7 embryos did not stain with the neurofilament antibody and their analysis is not included in this report. These specimens all had well formed inner ear structures and recognizable neuronal central and peripheral nervous system landmarks. Some of the artifacts present in the tissues examined could have resulted from inadequate penetration rate of the fixing solution into the tissues and/or to extraction due to a dilution effect of prolonged fixation. The samples were left in the fixing bags for months following passive diffuse (immersion) fixation. Moreover, after dissection the specimens were placed in buffered saline for shipment to the investigators. Ideally, specimens are fixed overnight following the exposure of the tissues to primary fixation in a ratio of 1:20 v:v tissue to fixing solution very rapidly with constant agitation and replenishing or replacement of the fixing solution immediately after it turns cloudy or bloody. The inner ear is one of the most difficult organs to reach and fix. Fixation artifacts could be decreased by using an additional fixation step that is possible with the present approved design: increase bag length and add a second clip that could hold a second fixing solution; after the initial fixation, remove clip and transfer egg to the new fixative. The egg shells could be cracked fully after initial fixation of the CAM to increase tissue exposure, but shorten time of tissue contact with fixative. Even under this arrangement, the four-bag arrangement should be considered because after mixing of the unused albumin and yolk following the opening of the shells, replenishing of the solution should be considered. This could be an excellent alternative to the ideal replacement of the exhausted fixative. Several reports prepared with data from ground controls were presented at scientific meetings (Davis *et al.*, *7th Midwinter Meet. of the Assoc. Res. Otolaryngol.*, 21:42, 1997; Fermin *et al.*, *American Society for Experimental Biology Meeting*, 1997) or published (Fermin and Martin, *Cell Mol Biol.*, 41: 213-225, 1995; Fermin *et al.*, *Cell Mol Biol.*, 41: 577-591, 1995). Additional results from the aims is are in press (Fermin *et al.*, *J Cell Vision*, 1997) and available from the PI's office upon request.

Methods used

Tissue Preparation: Tissues were received in buffered saline, examined, microdissected and post-fixed with 10% formalin, embedded in paraffin, and cut at 10 μ m thick. Each section was placed on a manila folder and saved. Sections with comparable inner ear structures from each stage above were placed on the same slide and stained together for anti-S100 β or anti-NF68/200. Staining sections from different experimental groups together eliminates inter-individual variability, and ensures that all groups receive equal treatment (Fermin and Martin, 1995).

Antibodies: Anti-S100 β (1:4000 dilution) was a rabbit polyclonal from East.acres Biologicals (lot 36 HT2-8) characterized as selective for the S100 β polypeptide that has less than 1% cross-reactivity with S100 alpha and no cross-reactivity with calmodulin (another calcium binding protein). The antibody reacts with human, bovine, rodent, and avian tissues. Anti-NF68/200 (1:200 dilution) was a monoclonal (clone 2F11) from BioGenex Laboratories. The antibody was characterized to label 68kD and 200 kD polypeptides of neurofilament and to stain neurons. No cross-reactivity was observed with GFAP, keratin, vimentin, or desmin.

Immunohistochemistry: In our laboratory we used procedures similar to those already published (Fermin and Martin, 1995). Briefly, all incubations were done at room temperature in a humidity chamber with tris buffer rinse in between: 1) 20 minutes in 1% hydrogen peroxide; 2) 10% goat serum for 20 minutes; 3) primary anti-S100 β rabbit IgG diluted 1:4000, or primary anti-NF68/200 diluted 1:200 in 2% Bovine serum albumin-Tris (BSA-TMS/HCL) buffer for 18 hours; 4) biotinylated goat-anti-rabbit IgG for 30 minutes; 5) streptavidin-horseradish peroxidase (Biogenex Laboratories, California) for 30 minutes; 6) 3'3'-diaminobenzidine tetrahydrochloride (DAB) for 5 minutes or 3-amino-9-ethylcarbazole (AEC); and 7) counterstain in hematoxylin.

Controls: Two negative controls were included: 1) omission or replacement of the primary antibody with an unrelated antisera, and 2) pre-absorption of antibody when the substrate was available. Two built-in positive controls exist in our preparations. First, the brain and cerebellum that contain positive reactive structures to the

antibodies in question are included with the inner ear preparation. Second, differences in the expression of the same a single antibody between different inner ear structures was always done on the same preparation. Color saturation (thresholding): The hues, saturation, and intensity (HSI) of reaction products was used to calculate the relative concentration of the antibody in the tissue sections in real time with a Videometric V150® (Oncor, Gaithersburg, Maryland). HSI permits a pixel-by-pixel analysis of the areas that contain a given color without regard to the density of the reaction. A numerical value was assigned to each color based on its position within a color cube, and subsequent identification of pixels with a similar threshold is done by the computer in frozen frames or in real time. The net results is that different colors of similar intensities are separated without filters (Fermin *et al.*, *J. Microscopy*, 167: 85-96, 1992), a property that favors histological examination (Fermin, *Microscopy Today*, 95-5: 16, 1995) and minimizes bias (Fermin and DeGraw, *J. Anat.*, 186: 469-481, 1995).

How does this fiscal year's progress affect future work on this task?

1) Preservation of inner ear tissues was just good enough to allow cytoskeletal component like the neurofilament to remain in the tissues, but there was evidence of cytosol coagulation, vacuolarization and membrane breaks in all three groups examined and most apparent at E10. E3 and E7 embryos did not stain with the neurofilament antibody and their analysis is not included in this report. These specimens all had well-formed inner ear structures and recognizable neuronal central and peripheral nervous system landmarks. Some of the artifacts present in the tissues examined could have resulted from inadequate penetration rate of the fixing solution into the tissues and/or to extraction due to a dilution effect of prolonged fixation. The samples were left in the fixing bags for months following passive diffuse (immersion) fixation. Moreover, after dissection, the specimens were placed in buffered saline for shipment to the investigators. Ideally, specimens are fixed overnight following the exposure of the tissues to primary fixation in a ratio of 1:20 v:v tissue to fixing solution very rapidly with constant agitation and replenishing or replacement of the fixing solution immediately after it turns cloudy or bloody. The normal staining pattern of inner ear tissues for anti-S100 β and anti-neurofilament were established in chickens (Fermin *et al.*, 1997). The embryonic development of chicken and quail differs only in their duration. Chickens hatch in 20 days, whereas quails hatch in 16 days. Thus, the inner ear structures of similar age embryos by days of incubation differs by 48 hours which in embryonic time could be an eternity. In fact, until critical periods of development are determined for each species, more quail ground controls need to be examined.

2) In E10 embryos, 77% of vestibular ganglion cells stained positive with anti-neurofilament in synchronous, whereas 90% of those counted from selected sections of the flight group were positive. This difference could be due to fixation artifacts. The surface area of the vestibular neurons of the synchronous embryos was 202 (SD = 30) square micra, whereas the surface area of the flight was 169 (SD = 40) square micra.

3) Anti-neurofilament reacted with its antigenic sites of the sensory inner ear structures at E10, but failed to recognize similar sites in E3 and E7 specimens. We expected positive staining in the E7 embryos because quail development is approximately 3 days ahead of the chicken development. In Leghorns, positive afferent fibers are observed in laboratory prepared E10 embryos.

4) The staining patterns of the neurofilament antibody was not always the same in all three groups when stained together, suggesting that there were marked differences in the mode of fixation and/or initial preparatory conditions for specimen in each group. We processed sections from all three groups together to avoid inter-individual variability. But despite this precaution, there was difference in the staining pattern of different sections from the same specimen suggesting that there was uneven diffusion and penetration of the fixing solution. Illustrations to compliment this report are available upon request.

This research does not seek to develop new therapeutic treatments for use at the 1-G Earth environment. The research will, however, provide invaluable information for better understanding the functioning of the vestibular (balancing and equilibrium) system in vertebrates. Even today, after decades of space exploration, astronauts suffer vestibular disturbances in microgravity despite intense and sophisticated training before space flights. The main reason for this is that at 1-G, certain conditions of the space environment can not be replicated for a long

period of time. Only long-duration adaptation to microgravity would provide the necessary training to diminish vestibular ocular conflicts that lead to motion sickness. Long-duration exposure to microgravity is only possible during space flights.

The results obtained will tell us whether microgravity affects the progression of normal development of processes that at 1-G are known to depend on stimulation aided by the force of the gravity vector. There are sufficient data published in peer-reviewed journals to indicate that otoconia found in the inner ear of vertebrates may influence the bearing load upon hair cells that lead to their depolarization and initiation of vestibular stimulus. However, we know nothing about the effect that microgravity may play in the development of otoconia when the animal is permitted to develop in microgravity from the time of conception.

The expected results may also help humans because motion sickness caused by variables other than lack of gravity afflicts millions in the 1-G environment of the Earth.

Questions remaining to be answered include: a) Can vertebrates developed in space without otoconia and function normally at 1-G when returning to Earth? b) Are the afferent fibers that convey otolith inputs to the brain affected? c) Are behavioral vestibular deficits induced by microgravity in space accompanied by reversible changes of the rewiring that induce the changes reversible? d) Are the changes compensated for in a time frame that permits functional readaptation in different environments?

In this project, proving or disproving the hypothesis is significant for the future of space exploration. A true hypothesis will alert humans to the effect of microgravity in the embryonic development of the inner ear vestibular apparatus. A false hypothesis will suggest that variables other than reduced gravity contribute to the development of motion sickness.

The results obtained from the Mir avian project are encouraging because they demonstrated that fertile eggs can be incubated and fixed in an orbiting space station and then returned to Earth via the space shuttle for scientific analyses. The ability to incubate eggs in space and eventually produce eggs in space is necessary to remove gravity from the developmental process. Fertile eggs produce at 1-G on Earth and brought to the space station via the shuttle are already 22 hours post fertilization and thus have experienced the gravity vector of the Earth environment. True lack of gravity as contributing variable for vertebrate development of avian embryos will be possible with eggs produced in space. Results obtained thus far permitted scientists to focus aims to maximize the science-return from future space experimentation.

The complexity of the space program and the need to mix politics with scientific design for successful completion of the SLM project suggests that cooperation between the partners of the planned international space station is a reality. Life science projects that evaluate alterations which lack of gravity may cause to vertebrates tissues and cell should be a priority on the list of things to do on the upcoming international space station. If changes that lack of gravity induce to tissues and cells were only chronic, traditional histological observations would suffice to demonstrate their severity. Unfortunately, many changes are transitory, reversible or simple sporadic and their scientific evaluation requires multidisciplinary approaches that incorporate cellular, molecular, and behavioral analyses

These results cover both the 1996 and 1997 task progress report.

FY97 Publications, Presentations, and Other Accomplishments:

Davis, S.F., Hara, H., Fermin, C.D., and Hoyle, G. "Substance P immunoreactive afferent inner ear terminals in knockout mice for p75 NGF is decreased." American Society for Experimental Biology Meeting. New Orleans, LA (April, 1997).

Davis, S.F., Hara, H., Hoyle, G., and Fermin, C.D. "Diminished expression of substance P immuno-reactive nerve terminals in knockout mice for p75 nerve growth factor." Proceedings of the 17th Midwinter Meet. of the Assoc. Res. Otolaryngol. 21:216 (1997).

Davis, S.F., Hartsfield, J.F., Hara, H., and Fermin, C.D. "Morphometric analysis of the statoacoustic and vestibular ganglia of the chick *Gallus domesticus*." Proceedings of the 17th Midwinter Meet. of the Assoc. Res. Otolaryngol. 21:42 (1997).

Fermin, C.D., Davis, S.F., Hara, H., Garry, R.F., Tenenbaum, S., and Martin, D.S. "Asymmetric expression of HSP and FOS after unilateral deafferentation." American Society for Experimental Biology Meeting. New Orleans, LA (April, 1997).

Fermin, C.D., Martin, D.S., and Hara, H. "Color threshold and ratio of S100 β , MAP5, NF68/200 GABA and GAD. I distribution in inner ear afferents." Cell Vis., 4(5) (1997).

Garry, R.F., Fermin, C.D., Kohler, P.F., Markert, M.L., and Hong, L. "Antibodies against retroviral proteins and nuclear antigens in a subset of idiopathic CD4+ T-lymphocytopenia patients." AIDS Res. Human Retrov., 12, 931-939 (1996).

Gatti, P.J., Fermin, C.D., and Garry, R.F. "Inhibition of HIV-1 production by hygromycin B, a selectively permeable translational inhibitor." AIDS (In Press).

Hara, H., Davis, S.F., Martin, D.S., and Fermin, C.D. "Neurofilament expression in normal and gentamicin treated chicks' inner ears." Proceedings of the 17th Midwinter Meet. of the Assoc. Res. Otolaryngol. 21:212 (1997).

Hara, H., Garry, R.F., Martin, D.S., and Fermin, C.D. "Sensory deprivation by deafferentation induces asymmetric expression of S100 β ." American Society for Experimental Biology Meeting. New Orleans, LA (April, 1997).

Makutonina, A., Norris, C.H., Plymale, D.R., Gatti, P.J., Fermin, C.D., Tensza, S.B., Meitzner, T.A., Montelaro, R.C., and Garry, R.F. "A synthetic peptide corresponding to the carboxy terminus of human immunodeficiency virus type 1 transmembrane glycoprotein induces alterations in the ionic permeability of *Xenopus leavis* oocytes." AIDS (In Press).

Makutonina, A., Voss, T.G., Plymale, D.R., Fermin, C.D., Norris, C.H., Vigh, S., and Garry, R.F. "Human immunodeficiency virus infection of T-lymphoblastoid cells decreases intracellular pH." J. Virology, 70, 7049-7055 (1996).

Merrogi, A.J., Marrogi, A.J., Ramesh, R., Robinson, W., Fermin, C.D., and Freeman S.M. "Tumor host interaction: Analysis of cytokines, growth factors and tumor infiltrating lymphocytes in ovarian carcinomas." Human Path, 28(3), 321-331 (1996).

Radhakrishnamurthy, R., Tracy, R.E., Fermin, C.D., Dalferes, E.R., Jr., and Bereson, G.S. "Immunohistochemical localization of proteoglycans in arteriosclerotic lesions." ASBMB/ASIP/AAI joint meeting. New Orleans, LA (1996).

Voss, T.G., Fermin, C.D., Levy, J.A., Bigh, S., Choi, B., and Garry, R.F. "Alteration of intracellular potassium and sodium concentrations correlates with induction of cytopathic effects by human immunodeficiency virus." J. Virology, 70, 5447-5454 (1996).

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Zimmermann, U., and Fermin, C.D. "Shape deformation of the organ of Corti associated with length changes of outer hair cell." Acta Otolaryngol, 116, 395-400 (1996).

Evaluation of Thermoregulation During Long Duration Spaceflight

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

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Flight Information:

Experiment ID: 95010311

Flight Assignment: SLM-1A (Spacelab-Mir)

Responsible NASA Center: Johnson Space Center

Task Description:

Impaired thermoregulation, which has been observed during exercise following bedrest, may have significant impact during space flight operations by decreasing exercise capacity and orthostatic tolerance. Impaired temperature regulation would be manifested as higher levels of core temperature for a given oxygen consumption as a result of an attenuated cutaneous vasodilatory reflex and sweating response. Two male crew members of the Mir 18 mission performed supine submaximal cycle exercise (20 min 40% and 20 min 65% preflight VO_2pk), once preflight (145 days) and 5 days postflight. Postflight, neither crew member completed the exercise protocol, stopping at 28-29 min of exercise. The core temperature (Ingestible Telemetry Pill) at test termination was similar (37.8°C) for both subjects pre- and post-flight despite the shorter test duration postflight. The slopes of the skin blood flow (laser Doppler)/core temperature relationship (Subj 1: 396 vs 214; Subj 2: 704 vs 143 Perfusion Unit/ $^\circ\text{C}$) and the sweat rate (dew point hygrometry)/core temperature relation (Subj 1: 4.5 vs 2.1; Subj 2: 11.0 vs 3.6 $\text{mg}\cdot\text{min}^{-1}\cdot\text{cm}^{-2}/^\circ\text{C}$) were reduced postflight. The core temperature thresholds for both sweating (Subj 1: 37.4 vs 37.6 ; Subj 2: 37.6 vs 37.6°C) and skin blood flow (Subj 1: 37.3 vs 37.5 ; Subj 2: 37.6 vs 37.7°C) were similar pre- to post-flight. For these two crew members, it appeared that heat loss responses were compromised after long-duration space flight.

During this funding period, data reduction was completed and a manuscript prepared and accepted in the *Journal of Aviation, Space, and Environmental Medicine*. The article is currently in press.

The results of this study will help to assess the potential for crew members to experience unexpected heat illness during strenuous activities (VA, inflight exercise) or during conditions of heat exposure (prolonged use of Launch and Entry Suit during emergency egress). Body heat storage after landing may contribute to postflight orthostatic intolerance and exercise intolerances. Development of specific procedures and countermeasures to prevent body heat storage during space flight (rehydration procedures, cooling garments, heat stress prediction equations) may prove useful in ground-based conditions in which heat loss responses are impaired (patients with inability to vasodilate appropriately such as those with hypertension, patients with impaired sweat responses, or workers or soldiers who wear impermeable clothing).

In this preliminary study to assess the potential for thermoregulatory impairment during space flight, countermeasures are not directly tested. However, countermeasures for heat stress experienced in the space program (e.g., liquid cooled garments, EVA suit life support system) have already been copied in Earth-based situations.

The results of this study will serve to test basic concepts of human temperature regulation. Specifically, the body temperature and sweating results obtained in this study will be entered into calculations of an Earth-based thermoregulation model (developed by the U.S. Army). We expect that since evaporation and heat convection may be impaired during space flight, the Earth-based predictions for body temperature responses will underestimate the degree of heat strain experienced by our crew members. Such results may help to confirm the role of sweating and convective heat loss in normal human thermoregulation.

Impaired thermoregulation during space flight will require the development of sensitive monitoring systems (non-invasive core temperature sensors, for example) and countermeasures to aid heat loss. These products may directly spawn spin-off products that may be used in the workplace; for example, the development of simple non-invasive core temperature monitoring systems, personal cooling systems with direct feedback from the body temperature responses, and/or more sensitive predictive models of heat strain. This new technology will also result in more comfortable and usable body-temperature monitoring and body-cooling systems.

FY97 Publications, Presentations, and Other Accomplishments:

Fortney, S.M., Mikhaylov, V., Lee, S.M.C., Kobzev, Y., Gonzalez, R.R., and Greenleaf, J.E. "Body temperature and thermoregulation during submaximal exercise after 115-day space flight." *Aviat. Space Environ. Med.* (In Press).

Effects of Weightlessness on Vestibular Development in Quail

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FY 1997 Funding: \$101,395

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Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

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Flight Assignment: Euro-Mir
Responsible NASA Center: Ames Research Center

Task Description:

The aim of this study is to expand ongoing research in rats which indicates that microgravity may alter synaptogenesis in the gravistatic information processing vestibular nuclei of the brain stem. The major problem with the rat model is the difficulty of obtaining animals continuously exposed to microgravity during embryonic, birth, and neonatal periods. Quail seemed to offer an opportunity to sidestep this problem and to allow at the same time an expansion of our findings in rats to another vertebrate species. Thus far, we have obtained only a fraction of the quail requested owing to a multitude of problems, not the least of which is the long incubation time (14 and 16 days) needed for our project which resulted in an increased mortality.

We proposed to analyze the central projection of the vestibular end organs such as saccule, lagena, and utricle and compare this with non-gravity sensing end organs such as the angular accelerometers of the semicircular canals. The method used is rapid diffusion of the lipophilic dye DiI in the vestibular nerve fibers after selective implantation of DiI crystals into the appropriate organs. This technique has been used extensively by us in the past on a variety of tissues (Bruce et al., 1997; Fritzsich, 1996; Fritzsich and Nichols, 1993; Fritzsich et al., 1993, 1997) and was previously successfully applied to an analysis of the vestibular connections in microgravity exposed rat embryos (Fritzsich and Bruce, 1995). However, in the microgravity exposed quail, we have encountered problems with this technique including improper diffusion and unspecific dye spreading (rather than following the fibers in the lipid bilayers). We suspect at the moment that this is due to incomplete fixation of the petrous bone.

To date we have received only two 14-day and one-and-a-half 16-day quail embryos exposed to microgravity and numerous control quail embryos. Once we found out that the DiI diffusion technique would not work in these immersion-fixed quail embryos, we changed our approach. We are now using immunocytochemistry of acetylated alpha-tubulin to analyze the innervation of the sensory epithelia. We have substituted the analysis of central projections using DiI for this analysis. The rationale behind this change is twofold:

- 1) No other techniques work in these poorly fixed specimens.
- 2) Any effects of microgravity on the innervation should show on either end of the nerve fibers connecting the sensory hair cells of the ear with the brain.

The data we have collected thus far on one quail embryo each exposed for 14 and 16 days, respectively, have not shown any obvious effect of microgravity. However, detailed quantification is now under way to study this issue more closely.

This research deals with a basic biological question. In conjunction with ongoing research in rats exposed during development to microgravity, this research could lead to a description of a critical phase during which the developing connections between the ear and the brain need a gravity stimulus to mature properly. This information could be crucial for any multi-generation space flight. Further ground based experiments using various hypergravity exposure times are now needed to consolidate the presumed effect of gravity on the reorganization of vestibular afferent fibers in the brain.

FY97 Publications, Presentations, and Other Accomplishments:

Bruce, L.L., Christensen, M.A., and Fritzschn, B. "Electron microscopic differentiation of directly and transneuronal transported DiI and applications for studies of synaptogenesis." *J. Neurosci. Meth.*, 73, 107-112 (1997).

Bruce, L.L., Kingsley, J., Nichols, D.H., and Fritzschn, B. "The development of vestibulocochlear efferents and cochlear afferents in mice." *Int. J. Dev. Neurosci.*, 15, 671-692 (1997).

Fritzschn, B. "On the role played by ontogenetic remodeling and functional transformation in the evolution of terrestrial hearing." *Brain, Behav. Evol.*, 50, 38-49 (1997).

Fritzschn, B., Farinas, I., and Reichardt, L.F. "Lack of NT-3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region specific fashion." *J. Neurosci.*, 17, 6213-6225 (1997).

Fritzschn, B., Silos-Santiago, I., Bianchi, L., and Farinas, I. "Neurotrophins, neurotrophin receptors and the maintenance of the afferent inner ear innervation." *Semin. Cell & Dev. Biol.*, 8, 277-284 (1997).

Fritzschn, B., Silos-Santiago, I., Bianchi, L., and Farinas, I. "The role of neurotrophic factors in regulating inner ear innervation." *TINS*, 20, 159-165 (1997).

Hypogravity's Effect on the Life Cycle of Japanese Quail

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$ 10,000

Solicitation: 93-OLMSA-06
Expiration: 1998
Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9306012
Flight Assignment: Euro-Mir
Responsible NASA Center: Ames Research Center

Task Description:

Exposing early stage chicken embryos to the space environment caused death, while chicken embryos launched into microgravity in later stages developed normally. Quail embryos have successfully completed embryogenesis in orbit, although success rate has been low compared to Earth-bound controls. Microgravity's role in the death of the embryos will not be known until a centrifuge is employed in orbit. Since avian eggs in microgravity were not turned, or were turned too infrequently or inadequately, the objectives of the current ground-based study were to determine the effects of frequency and orientation of turning on embryogenesis. Quail embryo viability was not affected by incubating eggs horizontally with daily rotation (4X) as compared to vertical orientation during incubation with hourly rotation. A decrease in frequency of egg rotation caused a concomitant linear decrease in hatchability for both quail and chicken eggs ($p < 0.01$). The hatchability of fertile chicken eggs were more adversely affected by lack of egg rotation than were quail eggs ($p < 0.05$). Gas exchange and nutrient distribution may occur more readily in unturned quail eggs as compared to chicken embryos because of the smaller quail egg size. The distance between the settling blastoderm relative to the shell surface is shorter for quail than for chicken embryos which may increase the probability of survival for quail as opposed to chicken embryos.

Five additional ground control groups were dissected May 26 through June 5, 1997 at Ames Research Center. Eggshell mineral analysis of these additional ground controls are currently in progress.

This research will help yield an understanding of the basic biological processes involved in avian development in microgravity. Results indicate that microgravity may be detrimental to the embryo's retrieval of calcium from the shell during development.

One of the long-term goals of developing the technology for avian development in space is to achieve a complete life cycle of Japanese quail (egg to egg) in microgravity. If this technology can be achieved, man will benefit during long-term space missions as the quail will serve as a source of food (meat and eggs) and as a companion animal for space travelers.

Effects of Gravity on Insect Circadian Rhythmicity

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Funding:

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Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$50,000

Solicitation: 94-OLMSA-01
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9401698
Flight Assignment: NASA-Mir-1B
Responsible NASA Center: Ames Research Center
Flight Hardware Required: Beetle Kit 1; Beetle Kit 2

Task Description:

The circadian timing system (CTS) coordinates temporal aspects of physiology and behavior. Disruptions in circadian timing not only adversely affect an organism's ability to respond to environmental challenges, but also decrease performance and contribute to psychological disorders in humans. Previous space flight experiments have shown that microgravity profoundly affects the circadian timing system of both vertebrates and invertebrates. Ground experiments have also shown that hyperdynamic fields produced by centrifugation influence the circadian system of several groups of living organisms. This research program will examine the effect of altered gravitational fields (microgravity via space flight and hypergravity via centrifugation) on the CTS of black-body beetles (*Trigonoscelis gigas*). We will examine changes in the endogenous period, mean level, and rhythmic characteristics produced by prolonged exposure to altered gravitational environments. Subsequent experiments will study the effects of altered gravity on the response of the insect CTS to: 1) light, 2) gravity pulses, and 3) 1-G via centrifugation during space flight. The data from these studies will significantly add to our understanding of the role of gravity on this fundamental physiological system. Further, these experiments on this simple biological system would likely suggest future experiments to increase our understanding of issues relating to biomedical problems of space flight.

During this phase of these studies, close communication between the investigators was maintained to ensure a smooth progress through the flight and ground experiments. Construction of the flight and ground support hardware was completed and the experiments were manifested for launch aboard STS-84 during May of 1997. Preflight procedures began in early April with the collection of the experimental subjects from the deserts of Turkmenistan by Dr. Alpatov. After a successful collecting trip, Dr. Alpatov returned to Moscow to make preliminary observations of the insects.

Dr. Hoban-Higgins traveled to Kennedy Space Center to set up the laboratory for the preflight screening of the experimental candidates. The laboratory was configured to allow for the simultaneous collection of activity data from 96 beetles and individual housing and maintenance of the insects and analysis of the collected data. Dr. Alpatov traveled to KSC with 195 adult *Trigonoscelis gigas*. The insects were allowed to acclimate to the laboratory environment for several days. Five days of activity data were collected from each beetle. These data were plotted and analyzed to determine the pattern and strength of the circadian activity rhythm of each potential flight subject. Drs. Hoban-Higgins, Alpatov, and Wassmer met and discussed each subject's data. Sixty-four prime and six backup flight candidates were identified.

On May 12, the 64 flight candidates were loaded into the flight hardware and data acquisition began. The six backup flight candidates were loaded into individual activity monitors. On May 13, all data was downloaded and examined. One flight candidate was replaced. The hardware was turned over for placement on the shuttle. STS-84 launched successfully at 4:07 am on May 15. The Beetle Kits were placed aboard the Mir space station and activated on May 18. The day-to-day management of the experiments was undertaken by Astronaut Michael Foale. The hardware is scheduled to be returned for data download and analysis aboard STS-86.

The remaining experimental subjects were transported to University of California, Davis where a delayed ground experiment was begun. The same protocols were followed as for the flight experiment, including a launch profile using a large diameter centrifuge.

Biological clocks are ubiquitous in living organisms. They are found in every eucaryote thus far examined. Although the first biological rhythms experiment was performed in 1729, it is only in the last 50 years that interest in the study of biological rhythms has grown rapidly. The CTS is responsible for the temporal coordination of physiological and behavioral functions both internally (i.e., with each other) and with the external environment (i.e., the 24-hour day). As such, the circadian timing system influences almost all physiological and behavioral functions. Humans have been thought to be unaffected by external light-dark cycles. However, we now know that sufficiently bright light will suppress human melatonin secretion and cause both entrainment and phase shifts of human circadian rhythms. This, coupled with the discovery of various chronobiologic disorders in humans has increased interest in circadian rhythm research. The CTS has been implicated in such phenomena as jet-lag, the problems associated with shift work, delayed sleep phase insomnia, and some forms of depression. Altered circadian rhythms are also seen in aged humans and laboratory animals. Alterations include changes in period and phase relationships and decreases in rhythm amplitude. These changes, coupled with our aging population, increase our need for an understanding of basic circadian physiology. Circadian function is affected by altered gravitational environments including the microgravity of space flight and hyperdynamic fields produced by centrifugation. Changes in the amplitude, period, waveform, phase relationships, and mean level of rhythmic variables have been reported. Alterations in circadian function can have deleterious effects upon an organism. Upon prolonged exposure to hyperdynamic fields, rhythmic functions recover back towards, but do not attain, precentrifugation levels. While microgravity is known to affect the CTS, the response of the CTS to prolonged space flight has not been examined. These studies will characterize the effects of long-term microgravity on circadian function in a simple organism, the black bodied beetle, *Trigonoscelis gigas*. These experiments could suggest future experiments on higher organisms (including humans) and increase our understanding of biomedical problems associated with space flight.

FY97 Publications, Presentations, and Other Accomplishments:

Hoban-Higgins, T.M., Alpatov, A.M., Wassmer, G.T., Rietveld, W.J., Fuller, C.A. "Response of insect activity rhythms to altered gravitational environments." Presented at the Annual Meeting of the International Society for Gravitational Physiology, Copenhagen (April, 1997).

Hoban-Higgins, T.M., Alpatov, A.M., Schnepf, T., Savage, P., Connolly, J., Hayward, E., Fenton, G., Hale, M., Higgins, J., Piert, S., Wassmer, G.T., and Fuller, C.A. "Beetles in space - long term monitoring of insect circadian rhythms on Nasa-Mir." Presented at the Annual Meeting of the Society for Gravitational and Space Biology, Charlotte, NC (October, 1996).

Sleep and Vestibular Adaptation

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Funding:

UPN/Project Identification: E639c

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1999

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$160,000

Flight Information:

Experiment ID: 9401663

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

Optimal human performance depends upon integrated sensorimotor and cognitive functions, both of which are known to be exquisitely sensitive to loss of sleep. Under microgravity conditions, adaptation of both sensorimotor (especially vestibular) and cognitive functions (especially orientation) must occur quickly and be maintained despite any concurrent disruptions of sleep that may be caused by microgravity itself or by the uncomfortable sleeping conditions of the spacecraft. It is the three-way interaction among sleep quality, general work efficiency, and sensorimotor integration that we will study in astronauts and cosmonauts participating in the U.S./Russian Mir Program from 1995 through 1997.

To record sleep, we will utilize a novel system called the Nightcap that we have developed and extensively tested on normal and sleep-disordered subjects. To perturb the vestibular system in ground-based studies, we will utilize "minifying" and reversing goggle paradigms that have been extensively studied in relation to plasticity of the vestibulo-ocular reflex. We will test the hypothesis that vestibular adaptation both provokes and is enhanced by REM sleep under both ground-based and space conditions.

NASA 4/Mir 23: During FY97, the Night Headband Monitor (NHM) was successfully used to monitor the sleep of the Mir 23 crews during a second 12-day block of preflight baseline data collection (BDC) and of the NASA 4 crew member during a second and third block of preflight BDC. Both crews launched successfully, and all three crewmembers recorded their sleep for three blocks of 12 nights each. One block of post-flight BDC has been collected for each crew member. The final block of post-flight BDC will occur early in fiscal 1998, completing the data collection for this crew.

Mir24: Two blocks of preflight BDC were carried out with the Mir 24 crew during FY97, prior to their launch and replacing of the Mir 23 crew. Because of the serious technical problems onboard Mir, sleep recording has been postponed and is now scheduled for early in fiscal 1998. At least two blocks of inflight recording are still planned.

Ground-based studies: A study of the effects of stimulation of the vestibuloocular reflex on subjective and objective measures of sleepiness was concluded during the year, and the results published (Leslie et al., 1997). This study examined the effect of optokinetic stimulation (OKS) on objective sleepiness as measured by the Multiple Sleep Latency Test (MSLT). The NHM was used in a novel way to perform MSLTs. Subjects came to the laboratory on two days. On the experimental day, subjects underwent 10 minutes of OKS, resulting in moderate motion sickness, prior to each of 4 MSLT trials. Although subjects in the OKS condition reported significantly more drowsiness than controls, this did not result in significantly reduced sleep latencies.

Recently, a further proposal has been advanced; not only does sleep enhance performance by preventing attentional lapses (a protective function), but it actually serves to promote the retention or consolidation of previously learned material (a conservative function). This second, stronger form of the theory is related to the hypothesis of vestibular-proprioceptive plasticity. It is supported by the preliminary findings of Karni and Sagi, which indicate that new visual discriminative learning is retained if and only if sleeping subjects enter rapid eye movement (REM) sleep stage. If neocortically mediated visual learning also proves to be REM sleep-dependent, then the plasticity-adaptation concept would have relevance not only to the space context but to plasticity enhancement in any context. Microgravity might then be viewed as a particularly potent test of the hypothesis that vestibular-mediated plasticity alters (and is altered by) REM sleep. Hence, we will test the hypothesis that vestibular adaptation both provokes and is enhanced by REM sleep under both ground-based and space conditions.

In our early time-lapse photographic and video studies, we established the strong temporal correlation between major posture shifts and sleep stage transitions. Under normal gravity, all humans make on average two major posture shifts per 90-minute sleep cycle: one tends to occur just before REM onset, the other at REM offset. During the intervening NREM and REM periods, major posture shifts are rare, though limb and head movements are observed. It is not known whether either the major posture shifts or head and limb movements are gravity sensitive, but it would not be surprising to find that they are. Indirect evidence comes from astronaut reports of bizarre sleep postures in space and of persistent limb elevations on awakening from post-flight sleep. Thus, gravity and microgravity may exert differential effects upon sleep posture, and these may, in turn, affect the quality and quantity of sleep and even of dreaming.

Since formulating the Activation-Synthesis Hypothesis of Dreaming in 1977, our group has developed a set of quantitative probes which measure formal aspects of dream cognition, including the illusion of movement. Our early work showed that dreaming subjects perceived themselves to be constantly moving through the dream space, a finding which we have recently confirmed and extended. In this and other recent work, we have shown that these dream features are REM-sleep based. One particularly interesting feature of dreamed movement (which we call "fictive" because it is illusory) is its "vestibular" content. This feature is prominent in reports and involves sensations of floating, swimming, sailing, flying, spinning, twitching, or turning, which dreamers generally regard as exciting or pleasurable. To our knowledge, this dream feature has never been quantified and therefore never measured in subjects before and after exposure to shifts in vestibular input such as those of microgravity.

As the vestibular system is initially perturbed by entry into microgravity, is the illusion of dreamed movement changed? Can this change be tracked as adaptation occurs? What new baselines are established under prolonged exposure? Finally, what are the sequences of changes when subjects reenter gravity? We see prolonged space flight in the Mir Laboratory as an ideal setting to assess vestibular adaptation via its effects upon the experience of fictive movement in REM-sleep dreaming.

This study will provide new information on sleep in space. It will provide the most extensive recording of sleep over prolonged exposure to microgravity yet obtained, the first collection of dream reports from space, and correlate changes in dream mentation, specifically fictive motor activity, with changes in sleep and adaptation to microgravity. It will also permit the correlation of any changes in REM duration or REM density with the process of adaptation to microgravity and, upon return to Earth, with readaptation to normal gravity.

FY97 Publications, Presentations, and Other Accomplishments:

Leslie, K.R., Stickgold, R.A., DiZio, P., Lackner, J., and Hobson, J.A. "Sleep and vestibular adaptation: Implications for function in microgravity." *J. Vestib. Res.* (In Press).

Leslie, K.R., Stickgold, R.A., DiZio, P., Lackner, J., and Hobson, J.A. "The effect of nauseogenic optokinetic stimulation on daytime sleepiness." *Archives Italiennes de Biologie*, vol. 3, 219-228 (1997).

Maier, E.A. Bizarreness and fictive movement in Nightcap mentation reports: Implications for activation-synthesis theory. (Thesis) Harvard University (1997).

Crew Member and Crew-Ground Interactions During NASA/Mir

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Funding:

UPN/Project Identification: E628

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 168,000

Flight Information:

Experiment ID: 9401628

Flight Assignment: NASA-5/Mir 24; NASA-6/Mir 25; NASA-7/Mir 26

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: MIPS System

Task Description:

During future space missions involving a space station or a trip to Mars, international crews will be engaged in complicated activities over long periods of time. A number of interpersonal issues likely to impact on these missions must be addressed in order to ensure healthy crew member interactions and optimal performance. A review of the literature of space analog studies on Earth, anecdotal reports from previous space missions, and the principal investigator's own work involving astronauts and cosmonauts have isolated crew tension, cohesion, and leadership as important interpersonal issues.

The objectives of this study are to measure and characterize changes over time in a number of important interpersonal factors, such as tension, cohesion, leadership role, and the relationship between space crews and monitoring personnel on Earth. These objectives will be assessed during the NASA/Mir missions by having both the crew members and personnel in ground control complete subscales from three standard mood and interpersonal group climate questionnaires: Profile of Mood States, Group Environment Scale, and Work Environment Scale. Along with a critical incident log and an experiences questionnaire, these measures will be completed on a weekly basis pre-mission, during the mission, and post-mission. By using an interrupted time-series analysis and a number of predicted correlations, a test of the hypotheses related to the objectives of our study will be made and discussed.

At present, we are mid-way through our study. We have complete data from 4 subjects who have flown in space, 31 different U.S. ground subjects at mission control in Moscow (an average of 5 respondents per week), and 14 Russian mission control subjects (an average of 7 per week). Overall, we have received 94% of the expected data. Missing data have resulted from sporadic glitches in reminding subjects to complete the

questionnaires and from dockings and other busy periods where subjects have not had time to perform a number of scientific activities.

Preliminary descriptive analysis of the data show that there is adequate variability within responses. For example, the full range of response options (from 1-5) on the Likert-type scales of the Profile of Mood States was given by both the American and Russian crew and ground subjects for all of the subscales, indicating that the subjects were critically evaluating their changes in mood from week to week.

In order to protect subject confidentiality and to enhance our chances of obtaining open, honest responses, we have agreed with our Russian co-investigators to delay performing hypothesis-driven data analyses until all 5 missions are completed. At that point, we will be able to randomize the order of missions and present our findings in terms of group averages that will protect the identity of individual subjects. Consequently, we have no results to present at this time that are related to our hypotheses.

In planning for future manned space missions involving international crews of men and women, it is important to prepare for the occurrence of interpersonal issues that might negatively affect the relationships of crew members and their ability to carry out mission goals. In recent results from space simulation studies (e.g., Antarctic expedition, EXEMSI, HUBES/Mir, and other multi-national simulator projects), anecdotal reports from space, and the author's work involving astronaut and cosmonaut communication in space and crew member interactions during the HUBES/Mir space simulation project, a number of interpersonal factors have been isolated that affect space crews and other small groups of people who must relate for long periods of time. These factors include interpersonal tension, crew cohesion, and leadership roles. These factors constitute the variables of interest in this study.

The interpersonal interactions of long-duration, multi-national space crews constitute a laboratory of small group behavior that tells us a great deal about ways in which groups of people on Earth can relate with a minimum of tension and improved cohesion when they are under stress. In addition, the ability of people from previously opposing political blocks to engage in complex activities, such as undertaking a space mission, serves as a model for international cooperation on Earth. Thus, this research project will teach us a great deal about ourselves and our ability to relate with one another despite cultural and political barriers.

FY97 Publications, Presentations, and Other Accomplishments:

Gushin, V.I., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., and Kanas, N. "Subject's perceptions of the crew interaction dynamics under prolonged isolation." (Abstract) *Aviat. Space Environ. Med.*, 68(7), 640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 160).

Gushin, V.I., Zapriska, N.S., Kolinitchenko, T.B., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., and Kanas, N. "Content analysis of the crew communication with external communicants under prolonged isolation." (Abstract) *Aviat. Space Environ. Med.*, 68(7), 640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 159).

Kanas, N. "Psychosocial findings from recent space simulation studies." (Abstract) *Aviat. Space Environ. Med.*, 68(7), 640 (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 157).

Kanas, N. "Psychosocial issues affecting Astronauts and Cosmonauts in space." (Abstract) *Aviat. Space Environ. Med.*, 68(10), 964. (From "1997 Abstracts of AsMA Scientific Sessions: Discussion Panel Abstracts", 1997, Abstract No. 555).

Kanas, N. "Psychosocial support for Astronauts." (Abstract) *Aviat. Space Environ. Med.*, 68(10), 964. (From "1997 Abstracts of AsMA Scientific Sessions: Discussion Panel Abstracts", 1997, Abstract No. 554).

Kanas, N. "Psychosocial value of space simulation for extended spaceflight." *Advances in Space Biology and Medicine*, 6, 81-89 (1997).

Kanas, N. "Crewmember and crew-ground interactions." Paper presented at the Phase 1 Research Program Interim Results Symposium, Johnson Space Center, Houston, TX. (1997).

Kanas, N. "Psychosocial issues affecting crews during long-duration international space missions." Paper presented at the 12th Man in Space symposium; Washington, D.C. (1997).

Kanas, N., Weiss, D.S., and Marmar, C.M. "Crewmember interactions during a Mir space station simulation." *Aviat. Space Environ. Med.*, 67, 969-975 (1996).

Kanas, N., Weiss, D.W., Marmar, C.M., and Grund, E.M. "Applicability of simulation studies to psychosocial issues in space." (Abstract) *Aviat. Space Environ. Med.*, 68(7), 640. (From "1997 Abstracts of AsMA Scientific Sessions", 1997, Abstract No. 158).

Kozerenko, O.P., Sled, A.D., and Salnitskiy, V.P. "Psychological support: Russian experience." (Abstract) *Aviat. Space Environ. Med.*, 68(10), 964. (From "1997 Abstracts of AsMA Scientific Sessions: Discussion Panel Abstracts", 1997, Abstract No. 556).

Cellular Mechanisms of Spaceflight Specific Stress on Plants

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Funding:

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Students Funded Under Research: 0
FY 1997 Funding: not available

Solicitation: 95-OLMSA-02
Expiration: not available
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9502026
Responsible NASA Center: Ames Research Center

Task Description:

The space environment affects the interplay among cell cycle controls, environment, and morphological and cell biological complexity. It is proposed that perturbations like reduced growth, and chromosomal and nuclear abnormalities observable in some space-grown plant samples and not in others, are due to a combination of factors including the biological "status" of the systems and the way things are grown and exposed to and, ultimately, the way they "experience" "space-specific stress." A long duration experiment on Mir in a BRIC using embryogenic cells of daylily in petri dishes as a model plant cell system will test a "space-environment specific stress to cells and tissues as related to environmental parameters and cell and developmental complexity" hypothesis. The experiment is designed to prove that: (1) protection from water stress will allow adaptation and normal cell division and development in prolonged microgravity; and (2) water stress will have serious negative effects on cell division and development if additional "mismatches" are imposed. The experiment will validate whether previously encountered chromosomal rearrangements are a result of adaptation to space stress. The question whether any effect of microgravity on gene transcription can be found and whether the responses of cells to space can be distinguished from other stress reactions will be a focal point of the methods used to evaluate material. Probing the precise cytological and biochemical nature of stresses to cells grown and adapted over a long period of space should disclose how space-related stress works. This findings will offer important information on the basic genetic mechanisms of adaptation to "stress" from space flight. Full testing of the hypothesis should facilitate growing of normal plants in space and reconcile different responses encountered by various investigators. It will also verify that space stress is a distinct kind of stress with an associated specific altered gene expression.

The hypothesis and conceptual framework will go far towards enabling one to reconcile all the different responses encountered in space and explain the many seeming discrepancies in results among various investigators. Moreover, if the hypothesis proves to be correct then space will afford a unique environment to

systematically dissect and study stress-related enzyme activities/protein degradation and gene transcriptional activities in response to microgravity in plant cells. It would provide opportunity to perform differential cDNA screening to monitor the steady state mRNA accumulation of known stress-related genes and of yet unknown genes involved in microgravity reactions. Researchers will be able to systematically study what g-unloading did over a protracted period and how it did it.

The work has progressed on two major fronts. These include: (1) scoring of the chromosomal status and profile of minuscule-sized plantlets recovered from the various dishes of the Mir-grown material as soon as possible after recovery of the flight package on earth; and (2) rearing recovered embryos into so-called plants resuscitated from the Mir spaceflight exposure with the view of monitoring their performance and chromosomal status as they progress through various degrees of successive growth and recuperation. Each of these efforts is being carried out with detailed evaluation of the resuscitability of the embryos.

We are beginning to accumulate information that attests to our suggestion that the embryos and small plantlets that were recovered from the Mir Mission had undergone considerable stress in the course of their exposure to the space environment. The first indication of this was the general visual evaluation of the embryos under low-powered dissecting microscopy indicating that they had experienced substantial challenge during flight. This was further verified by the observation that the epidermal surfaces of recovered materials examined via scanning electron microscopy (SEM) on materials collected at recovery disclosed with certainty that the many of the specimens possessed epidermises with cells in varying degrees of desiccation.

Thus far, the greatest extremes of this desiccation have been observed in the smallest embryo units under/in conditions of the category designated as "non-optimized." "Non-optimized" means that the embryo initial units were "small" and thus on the basis of the hypothesis being tested, they were expected to be less able to withstand the rigors of space flight than those units that were larger, namely those units in position number 4 in the BRIC canisters. The epidermal surfaces of the larger units, while also desiccated, were far less shriveled than that of the smaller units, whether "optimized" or not.

Additional work using scanning electron microscopy of the flight-exposed units indicates that the larger units tolerated the space environment with less apparent "stress" than those embryos that were considerably smaller and younger in developmental stage. The larger units were not only larger at the outset of the experiment (and hence, but not surprisingly, larger at the end of the experiment), they were also healthier and showed less desiccation in their epidermises, and indeed all parameters. The tentative conclusion from these observations is the larger the size of an embryo "unit" [of daylily at least] exposed to space, the least likely it is to suffer drastically from space exposure; the smaller the unit, the more likely it is that it will show "signs" of stress. What has just been said relates to the epidermises of the recovered embryos. Representative roots of these embryos, when present, have also been scanned, and these too vary in terms of their status or vigor at recovery. Roots on the larger units (again those in position 4 of the BRIC canister) were better maintained than those on smaller embryo units. This suggests that the root system is better able to maintain itself when there is a larger amount of pre-flight exposure growth. The units are larger, more vigorous, and hence better suited to last for a longer duration than those smaller embryo units that have less reserve foodstuffs on which to draw during their protracted exposure to flight.

The science results which were anticipated from this multi-faceted experiment were expected to lead to new strategies which will allow systematic manipulation, dissection, and exploitation of the effect of gravity on plant development, growth, and biochemistry using aseptically cultured plant cells and tissues. Such understanding of the behavior of *in vitro* systems under extended space flight conditions will eventually directly benefit agricultural, horticultural, and forestry industries and biotechnologies which depend upon plant growth for their products.

The genetic information in a plant, that is the plant genome packaged in chromosomes, is best viewed as in a state of flux with many changes occurring during development and reproduction. Variation can occur in response to various internal and external influences and stresses. We have encountered significant changes in the nuclei and chromosomes of cells of space-grown somatic embryos, space-generated roots on tissue culture-derived

plantlets, and space-generated roots on cloned seedlings. It is important to know how widespread or general a phenomenon these changes in the genetic information are and how much digression from the "norm" is tolerated by developing plant systems. We need to understand the precise nature of the various kinds of change so that we can probe how and why they came about. A full understanding of these variations is of great importance if we are to grow plants efficiently in space and to use them to study basic aspects of gravity on plant function, metabolism, and growth. Changes may occur in the highly repetitive fraction of the genome and are probably limited to specific chromosomal sites. One likely function of these genomic variations is the generation of a radical, but limited, reorganization of the genome in response to stress perhaps in order to increase the pool of variability from which new (better adapted?) types of cells can be selected or emerge. On this view, there would be adaptive value to these reorganizations in microgravity.

FY97 Publications, Presentations, and Other Accomplishments:

Krikorian, A.D. "Plant cells in space: What have we learned?" *ASGSB Bull.*, 11 (no. 1), 3 (1997).

Anticipatory Postural Activity During Long-Duration Space Flight

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FY 1997 Funding: not available	
Joint Agency Participation: U.S./Russian Space Agency	

Flight Information:

Experiment ID: 9400423
Flight Assignment: SLM-1A, Mir-21/NASA-2
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: Foot Pressure Boots, Belt-Pack Amplifier System

Task Description:

The proposed project is designed to investigate the fundamental contributions of cutaneous and proprioceptive information in maintaining in-flight neuromuscular activation and postflight postural equilibrium. Developing appropriate in-flight countermeasures to maintain neuromuscular activation and minimize muscle atrophy will reduce the postflight postural control problems experienced by many crew members. The primary objective is to determine whether in-flight foot sensory input can be used to maintain 1-g neuromuscular activation patterns associated with arm movement. The secondary purpose is to determine the effect of long-duration space flight on postflight postural control responses and postural stability during arm movement.

The experimental protocol involves the crew members raising their arms as rapidly as possible before, during, and after flight. The inflight testing consists of four arm-raising conditions that are designed to vary the degree of foot sensory input. Arm movements are completed while the subject freefloats, freefloats with the addition of foot pressure, is secured passively at the feet to the Mir or Shuttle's support surface with Velcro™, and while connected via bungee cords to the support surface. Electrical activity (EMG) from selected arm, trunk and leg muscles and arm acceleration is monitored. Muscle-activation latencies referenced to arm movement initiation are obtained and temporal muscle activation patterns developed for each experimental condition. In this way any changes in the neuromuscular activation characteristics associated with the experimental conditions are detected. During preflight and postflight testing, subjects perform the arm raises while standing on a force plate in order

to obtain ground reaction forces and center of pressure (COP) measures. Body segment kinematic measures are also obtained. These measures enable determination of the degree of postflight postural instability associated with voluntary arm movement. As hypothesized, lower limb neuromuscular activity normally preceding arm movement during 1-G movements is eliminated while subjects freefloat but is restored when foot sensory input is available. It has also been shown that postural instability, as measured by excursions of the COP, increases relative to preflight values. For an in-flight experiment, the project has proceeded smoothly, flying on STS-63, STS-71 (ground-based portion of protocol), Mir 18, Mir 19, and Mir 21. It is currently flying aboard Mir as part of the Russian Science Program. Data analysis is proceeding steadily and several preliminary reports have been made. The results are corresponding to the hypotheses.

To date, 9 subjects have completed the inflight portion of the experiment and 11 subjects have completed the ground-based experimental conditions. Of these subjects, 8 have flown long-duration missions.

Data analysis of the inflight data has included the following: (a) average arm accelerations have been normalized to peak accelerations obtained in the freefloating arm raise condition and comparisons between the various inflight conditions have been made; and (b) filtering, rectifying, and averaging of the EMG data, amplitude, and temporal normalization of the data files, determination of muscle activation onsets (relative to arm movement initiation), and measures of peak activation have been developed.

Data analysis of the ground-based data has included the following: (a) average arm accelerations have been normalized to peak preflight values and comparisons between pre- and postflight accelerations have been made; (b) filtering, rectifying and averaging of the EMG data, amplitude and temporal normalization of the data files, determination of muscle activation onsets (relative to arm movement initiation) and correlations between pre- and postflight waveforms have been developed; and (c) COP measures have been temporally synchronized with arm movement initiation, the anterior-posterior and medial-lateral components of the COP were then separated and phase portraits (position vs. position velocity) of each component developed. Since the COP represents the amount of thrust applied to the plate, the phase portraits of the COP directly represent the postural control strategies used by the subjects to complete the arm raise task. Changes in COP phase portraits associated with space flight therefore represent changes in motor control strategies.

For the inflight portion of the study, it has been shown that the use of static foot pressure during freefloating arm movements results in increased neuromuscular activation compared to freefloating arm movements performed without foot pressure.

The above scientific evidence has supported the decision to develop a prototype variable pressure boot which mimics the pressure on the soles of the feet experienced during walking, running, and jumping. This prototype is now ready for testing to determine the neuromuscular activation patterns associated with the patterns of foot pressure provided by the boot.

For the ground-based portion of the study, it has been shown that postural control strategies used to maintain equilibrium after space flight (and their associated neuromuscular activation patterns) are modified relative to preflight control parameters. These changes are also associated with a decrease in arm acceleration thus indicating that in spite of lower arm accelerations (a decreased perturbing force), subjects exhibit increased postural instability after space flight. This is the first systematic demonstration that postural control associated with voluntary upper limb movement is compromised following flight.

Using the information gained from both the inflight and ground-based data, a 7-segment dynamical computational model of a human has been completed and has been used to perform "desktop" experiments. The results of these experiments were presented at the International Society of Biomechanics in Sport Symposium, Denton, Texas in June 1997. This model uses algorithms which enable it to predict optimal solutions to a variety of movement tasks. A manuscript providing a detailed account of the inflight portion of the experiment is currently under review.

We have been able to keep the project on schedule. The work completed this year will enable us to develop peer-reviewed manuscripts in the near future and hopefully continue the development of the foot pressure boot. Future work will proceed at a slower pace than in the past due to cuts in the workforce.

This project provides information about the magnitude of postural control decrement that is associated with space flight. It also seeks to understand the role of cutaneous and proprioceptive input in the generation of neuromuscular activation. The responses observed in returning crew members have features in common with Parkinson patients who have performed arm raising tasks. Thus, this project may be able to provide information which can further our understanding of particular disease states.

One of the goals of this project is to validate the concept that the sensory input associated with foot pressure increases lower limb neuromuscular activation relative to conditions without foot pressure. A prototype variable foot pressure boot which mimics the pressure patterns associated with walking, jumping, and running has already been developed. It is anticipated, that in addition to serving as an inflight countermeasure designed to attenuate muscle atrophy, a version of the pressure boots will be used with bedridden patients. In both Austria and Russia, foot pressure is routinely applied with great success to a variety of bedridden populations. The dynamic computational model will be used to predict optimal movements solutions for a particular task. Since the model allows for the changing of initial conditions (e.g., 20% loss of ankle muscular strength, limb amputation, restricted range of joint motion), it will be used to predict optimal movement outcomes for a variety of patient populations. Therapists can then design rehabilitation programs designed to reach the optimal functional state that can be achieved by a particular patient.

This project has the potential to increase our understanding of the processes whereby sensory input results in neuromuscular activation. It is suggested that many of the processes that contribute to muscle atrophy on Earth (i.e., muscle disuse, lack of sensory input) also contribute to the atrophy associated with space flight. It is anticipated that foot pressure will be regularly used to attenuate lower limb muscle atrophy and maintain the functional state of proprioceptive reflex loops in bedridden patients. Dynamic computational models will eventually be used to visualize and predict movement outcomes for both patient and athletic populations.

In addition to the benefits listed above, the dynamic computational modeling and devices which provide controlled patterns of sensory input will be integrated into virtual reality environments. Adding sensory input to the virtual environment will dramatically improve the fidelity of these environments for use as training tools. Computational models will eventually be introduced into the virtual environments to "discover" optimal solutions to a variety of tasks. Information gained from these predicted optimal outcomes will be incorporated into training protocols.

FY97 Publications, Presentations, and Other Accomplishments:

Kalakanis, D., Layne, C.S., Abraham, L.D., and Bloomberg, J.J. "Influence of external forces on the control and performance of a minimum time shoulder flexion task." Presented at the International Society of Biomechanics in Sport Symposium, Denton, TX (June, 1997).

Magnetic Resonance Imaging After Exposure to Microgravity

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Solicitation: 94-OLMSA-01
 Expiration: 1997
 Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9401586
 Flight Assignment: NASA-Mir-1B
 Responsible NASA Center: Johnson Space Center

Task Description:

Our measurements on the crew of SL-J demonstrated significant muscle-specific atrophy after only eight days in weightlessness. Our published bedrest studies have documented the degree of expected atrophy after four months of disuse. We will repeat these muscle measurements on the long-duration missions of Shuttle/Mir to determine the degree of protection provided by the Mir exercise program. Our bedrest studies have shown that when normal subjects are put in bedrest, partially unloading the spinal column, significant intervertebral disc expansion occurs. This expansion reverts to normal shortly after reambulation following bedrest lasting days to a few weeks. Longer duration bedrest (17 weeks) however, results in some residual expansion that remains for some time following reambulation. We have shown that eight days of weightlessness (SL-J) does not result in residual expansion 24 hours after landing. We speculate that disc expansion during flight may be causally related to the back pain reported to occur during flight and that longer duration space flight will result in residual disc expansion that may pose some risk of disc damage during the landing and early post-flight period. This disc expansion with back muscle atrophy may be causally related to the back pain experienced after long-duration space flight. Several space experiments have documented altered hematopoietic activity which may be related to cellularity changes in the bone marrow. This research will measure the intervertebral disc cross-sectional area,

muscle volumes and spinal bone marrow cellularity of the crew members before and after the Shuttle/Mir flights.

Preflight and postflight measurements have been completed on NASA 2 and NASA 3. Postflight testing on NASA 4 and NASA 5 continues. A single preflight MRI measurement on NASA 6 was accomplished prior to launch. All preflight and postflight measurements on the Mir 22 crew have been completed, while postflight measurements on the Mir 23 crew continue. The data analysis is complete on NASA 2 and NASA 3; analysis of NASA 4 and Mir 22 is in progress.

Space flight measurements have documented that significant bone and muscle atrophy occurs during weightlessness. Knowledge of the extent and temporal relationships of these changes in the individual bones and muscles is important for the development of effective countermeasures. The losses during space flight are believed to result from the reduced forces on the musculoskeletal system. Analogous to space flight, inactivity in 1-G will cause bone and muscle loss. The loss of bone and muscle with aging occurs in both men and women, resulting in a significant public health problem. Although the exact cause of bone and muscle loss with aging is not understood, one important risk factor is disuse. Men and women become less active as they grow older, and that may play an important role in the elderly and in patients immobilized for medical reasons. In addition, muscle atrophy is an important component of many disease states as well as aging; therefore understanding the role of disuse versus other causes is important for elucidating the physiological mechanisms of muscle atrophy. The relationship of muscle atrophy to muscle performance is not well understood. The LMS flight will examine decrements in muscle performance with measurements of muscle-specific atrophy.

Back pain is a common health problem. There are several causes for this complaint and it often involves the intervertebral discs. Bedrest is frequently recommended as a component of patient management. Our studies demonstrated that overnight or longer bedrest causes expansion of the disc area, reaching an equilibrium value of about 22% (range 10 - 40%) above baseline. In space, where the external mechanical loads are greatly reduced, the disc probably expands significantly. These changes, which are rapidly reversible after short-duration flights, may be an important consideration during and after long-duration missions or bedrest on Earth, i.e., long duration disuse may alter disc physiology. Also, this change in the disc size may be causally related to the back pain experienced during space flight.

FY97 Publications, Presentations, and Other Accomplishments:

LeBlanc, A. "Bone and muscle atrophy during space flight and simulated microgravity." TAMU Chapter of American Nuclear Society, College Station, TX (April 8, 1997).

LeBlanc, A. "Experiment 586-magnetic resonance after exposure to microgravity." Phase I Research Symposium. Lyndon Johnson Space Center, Houston, TX (August 5 - 8, 1997).

LeBlanc, A. "Investigation of effects of microgravity on skeleto-muscular system." International Space University, Rice University, Houston, TX (July 24, 1997).

Oganov, V., Schneider, V., Bakulin, A., Polyakov, V., Veronin, L., Morgun, V., Shackelford, L., LeBlanc, A., Murashko, L., and Novikov, V. "Human bone tissue changes after long-term space flight: Phenomenology and possible mechanics." 12th Man In Space Symposium, Washington, DC (June 8 - 13, 1997).

Shackelford, L., Feiveson, A., Spector, E., LeBlanc, A., and Oganov, V. "Prediction of femoral neck bone mineral density change in space." 12th Man In Space Symposium, Washington, DC (June 8 - 13, 1997).

Avian Blood Formation in Space

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Funding:

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 Initial Funding Date: 1995
 Students Funded Under Research: 2
 FY 1997 Funding: \$ 10,000

Solicitation: 93-OLMSA-06
 Expiration: 1998
 Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9306014
 Flight Assignment: Euro-Mir
 Responsible NASA Center: Ames Research Center

Task Description:

The initiation and maturation of the vasculature is an essential process during embryonic development. Previous studies have shown that birds which, as embryos, were exposed to microgravity during space flight, exhibit developmental anomalies which might be related to (or caused by) delayed or improper vascular development. For example, the area vasculosa, the region of blood island formation and the forerunner of the chorioallantoic membrane, was reportedly deformed in some quail embryos that had developed during space. Also, other studies have shown that specific cellular events which may be key to neovascularization, such as directed cell migration, homing, intracellular signal transduction, enzymatic activities, and the metabolism of extracellular matrix proteins seem to be affected by microgravity.

Based on these studies, we hypothesize that the developmental anomalies observed in the past might be related to or caused by delayed or improper vascular development. Specifically, we hypothesize that at a given developmental stage, such vascular abnormalities will be manifested by altered capillary density and changes in the expression of subendothelial extracellular matrix (ECM) proteins. In testing this hypothesis, we will analyze quail chorioallantoic membrane (CAM) and adrenals at various stages of development. We propose these particular tissues as specific locations at which two different modes of vascular development occur: vasculogenesis in the adrenal (i.e., the *in situ* development of blood vessels from local mesenchymal vascular precursor cells), and angiogenesis in the CAM (i.e., development of new blood vessels by endothelial cell migration from pre-existing vessels).

The specific aim of this proposal is to test our hypothesis. The methodological approach is dictated by the constraints of the tissue preservation method used in space. We propose to first semi-quantitatively assess whether there is indeed a change in the pattern of vascularization during and after exposure to microgravity in space. If indeed this is the case, we propose to proceed beyond the mere descriptive phase and to address a

mechanistic question by analyzing the temporal and spatial expression of angiogenic growth factors and their receptors.

Specifically, we will initially count, in histological preparations, vessels and immunostain endothelial cells with specific antibodies (anti- vWF and QH1). The extent of ECM protein deposition will be assessed by immunohistochemistry and correlated with the degree of vascularization, using computer-based image analysis. Also, the cellular source for ECM proteins will be assessed by *in situ* hybridization. If indeed we find significant differences in the pattern of neovascularization between ground and space animals, we hypothesize that such differences might be related to altered expression of angiogenic/vasculogenic growth factors (e.g., FGF or VEGF) and/or their receptors. If the first hypothesis is verified, we will use the available tissues to probe, by immunohistochemical and molecular biological means, for the expression of aFGF, bFGF, VEGF, and their respective receptors. As controls we will use the matched time delayed and synchronous animals as provided by the US/Russian team.

This study is, to the best of our knowledge, the first one which specifically proposes to analyze the effects of microgravity on avian vascular development. Since this study is the first of its kind, we believe that the outcome (whatever the results may be) will significantly contribute to our scant understanding of the effects of microgravity and space flight on embryonic vascular development.

A substantial number of useful samples were returned from the Mir 21 Mission in September 1996. In addition, various laboratory and controls were performed on the ground which will be helpful for sorting out the contributions, if any, of temperature, rotation and simulated launch conditions.

Preliminary evaluation of the flight samples indicates that the development of the vasculature in the chorioallantoic membranes (CAMs) in the flight samples, as inferred from vessel density and vessel size, seems retarded, as compared to the laboratory controls (LAB-1). This includes both diminished numbers of small vessels for the day 14 and 16 embryos as well as a delay in the time point of the peak of angiogenic activity around day 10. However, given that only 3 time points (days 7, 10, 14) are available for this critical period, no exact time-course can be made, at this point.

There was no profound effect of the "flight-simulating conditions" on the development of the CAM in the synchronous controls (SYNCH-1). In this control, however, the eggs had not been exposed to some of the critical components of a simulated launch, but were incubated at an elevated temperature, comparable to that on board Mir. Our preliminary data indicate that the development of the vascular architecture (in terms of vessel number and complexity) in both the CAMs and the adrenals appears to be somewhat accelerated by comparison to that of the laboratory controls.

Preliminary analysis of samples from SYNCH-2 and SYNCH-3, in which the eggs were exposed to the mechanical forces (g-load, vibrations, etc.) of a simulated launch, indicates that vascular development in the CAM appears to be impaired, resembling the pattern observed for the synchronous controls of Mir 18. *viz.*, the retardation in the vascular development observed in the samples from SYNCH-2, is probably due to the lack of egg rotation in SYNCH-2 vs. 3 rotations/day in SYNCH-3. The elevated temperature in the LAB-3-HI controls is indeed reflected in an accelerated vascular development.

Preliminary Conclusions and Future Studies

At this time, we continue to fully evaluate all the samples from Mir 21 and the diverse controls according to our original plans. Our provisional analysis indicates a retardation is concomitant with possible impairment of the formation of the vasculature in the CAM of flight samples. The different laboratory and synchronous controls suggest complex effects of the various parameters on vascular development. Elevated temperature accelerates, lack of rotation retards vascular development in the CAM, but does not otherwise interfere with angiogenesis. By contrast, some of the mechanical forces to which the eggs are exposed during the simulated launch seem to impede the regular development of blood vessels at the time of the "angiogenic burst" and hence may be related to the premature deaths of many embryos observed on Mir 21 and some previous missions.

Further analysis of the samples will focus on finishing the evaluation of vascular development in the CAMs by light microscopy, immunohistochemistry (ECM proteins, growth factors), and by RT-PCR. Furthermore, we will complete a detailed assessment of the vascularization in the adrenals.

The goal of this research is to understand the effects of microgravity on vascular development. As such, our studies are not primarily aimed at understanding specific diseases. However, as with all types of microgravity research, the effect of gravity on a particular phenomenon can only be assessed in the absence of this force. If, as we hypothesize, microgravity impairs vascular development, our research might ultimately disclose mechanisms involved in vascular diseases.

The goals and methodologies employed in this research are designed to contribute to our understanding of basic scientific processes in space biology. Specifically, we propose to investigate the effects of space flight on cellular and molecular mechanisms, factors, and their cognate receptors, which are involved in the early development of blood vessels. Since all embryonic/fetal development as well as the well-being of adult organisms is dependent on proper functioning of the vasculature, the studies are of fundamental interest both from the basic science vantage point as well as for space physiology. Specifically, our studies could have far-reaching implications for the prospects for "normal" embryonic development in space.

Correlation of Disconjugate Eye Torsion with the Time Course of the Space Adaptation Syndrome

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

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Joint Agency Participation: ESA

Flight Information:

Flight Assignment: Euro-Mir-95

Responsible NASA Center: Johnson Space Center

Task Description:

Three astronauts underwent preflight, inflight, and postflight testing of spontaneous ocular torsion and ocular counterrolling (OCR), reflexes governed by the gravity-responsive otolith organs in the inner ear. One astronaut, subject A, had a 30-day space mission on Euromir '94 and was examined monocularly with SensoMotoric Instruments' video-oculography (VOG). The other two astronauts, subjects B and C, were studied with a binocular VOG and flew a 180-day mission on Euromir '95.

In space, spontaneous eye torsion in the upright position was found to be substantially offset from baseline Earth-based recordings in all three subjects for the duration of the flights. In addition, the binocular studies showed a marked torsional disconjugacy. On return to Earth, offset and torsional disconjugacy persisted for many days.

OCR in response to 30 degree right and left tilt was examined preflight and postflight. Compared to preflight, subject A showed reduced OCR immediately postflight, which gradually approached but did not achieve the preflight values over 13 days postflight. The adaptation of ocular torsion in space in one astronaut and not in the other two, and slow adaptation postflight, may reflect the lack of visual feedback and the open loop nature of the otolith-ocular torsion reflex.

Data analysis was completed on all videotapes of three astronauts, consisting of several preflight baseline data collections, all inflight recordings, and postflight recordings made every other day from postflight Day 0 to postflight Day 13.

A manuscript was prepared documenting this experiment and was submitted to the *Journal of Vestibular Research* for peer review. It was accepted and is in press at the present time.

Long-term postflight studies were performed on the two astronauts who participated in the Mir '95 mission, to complement the long-term study earlier conducted on the Mir '94 astronaut. The FY 1997 studies were performed in our laboratory at the University of California at Santa Barbara.

The space motion sickness aspect of this study was unable to be performed as none of the three astronauts experienced space motion sickness during their missions. However, considerable new information was obtained regarding the inner ear otolith organs, a complex system not heretofore well defined. Findings included the instability of the ocular torsion reflex in hypogravity and the disconjugacy indicating the two eyes have separate controlling influences. This study emphasizes the importance of having an external baseline, i.e., the preflight position of the eyes, to measure the changes occurring in space flight and following return to Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Diamond, S.G. and Markham, C.H. "The effect of space missions on gravity-responsive torsional eye movements." *J. Vestib. Res.* (In Press).

Gushin, V.I., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., and Kanas, N. "Subject's perceptions of the crew interaction dynamics under prolonged isolation." (From "1997 Abstracts of AsMA Scientific Sessions" 1993, Abstract No. 160). *Aviat. Space Environ. Med.*, vol. 68, 640 (1997).

Gushin, V.I., Zaprisa, N.S., Kolinitchenko, T.B., Efimov, V.A., Smirnova, T.M., Vinokhodova, A.G., and Kanas, N. "Content analysis of the crew communication with external communicants under prolonged isolation." (From "1997 Abstracts of AsMA Scientific Sessions," 1993, Abstract No. 159). (Abstract) *Aviat. Space Environ. Med.*, vol. 68, 640 (1997).

Kanas, N. "Psychosocial findings from recent space simulation studies." (From "1997 Abstracts of AsMA Scientific Sessions," 1993, Abstract No. 157). (Abstract) *Aviat. Space Environ. Med.*, vol. 68, 640 (1997).

Kanas, N. "Psychosocial value of space simulation for extended spaceflight." *Adv. Space Biol. Med.*, vol. 6, 81-89 (1997).

Kanas, N. "Crewmember and crew-ground interactions." Phase 1 Research Program Interim Results Symposium, Johnson Space Center, Houston, TX (1997).

Kanas, N. "Psychosocial issues affecting crews during long-duration international space missions." 12th Man in Space symposium, Washington, DC (1997).

Kanas, N., Weiss, D.S., and Marmar, C.M. "Crewmember interactions during a Mir space station simulation." *Aviat. Space Environ. Med.*, vol. 67, 969-975 (1996).

Kanas, N., Weiss, D.W., Marmar, C.M., and Grund, E.M. "Applicability of simulation studies to psychosocial issues in space." (From "1997 Abstracts of AsMA Scientific Sessions," 1993, Abstract No. 158). (Abstract) *Aviat. Space Environ. Med.*, vol. 68, 640 (1997).

Markham, C.H. and Diamond, S.G. "The effect of long-term hypogravity on the eye torsion responses governed by gravity receptors in the inner ear." 122nd Annual Meeting of the American Neurological Association (September 29, 1997).

Human Circadian Rhythms and Sleep in Space

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No Co-Is Assigned to this Task

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$135,000

Flight Information:

Experiment ID: 9401639

Flight Assignment: NASA 4/Mir 23, NASA 5/Mir 24

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: IBM "Thinkpads" and floppy diskettes

Task Description:

The aims of this study are to evaluate the sleep, mood and activation, body temperature, and performance of crew members involved in long-duration Mir space missions in microgravity. Because of time constraints for our experiment, we cannot study every day of the mission. Instead, three twelve day measurement blocks will be studied (early, middle, and late mission). This will, we hope, allow determination of the period length at which circadian rhythms in subjective activation and body temperature run, in order to detect "free-running" behaviors and to determine their consequences in terms of the patterning of daily levels of sleep quality and duration, mood, activation, and performance efficiency over the mission. Sleep will be evaluated by computerized sleep diaries (pre- and post-sleep); circadian rhythms by oral temperature, mood and subjective alertness (five times per day); and performance by verbal reasoning and serial search tests given one time per day.

FY97 saw our experiment performed by the NASA 4 astronaut Mir from January through May, 1997. The subject performed three data blocks during his time aboard Mir. We received very good data from this subject and are still in the process of analyzing all it. The Mir 23 cosmonauts also performed our experiment during FY97. We have received one data block from them and are still analyzing this data also. We will receive the rest of their data later this year. Despite the accident which disabled the Spektr module, the cosmonauts did have access to our equipment and were able to perform another data block. The Mir 24 cosmonauts are also scheduled to perform our experiment during FY97.

Life on Earth has developed to be in tune with cycles of daylight and darkness that stem from our planet's 24h rotation. Like most other animals, human beings have a biological clock inside the brain which acts as a timekeeper. For diurnal creatures like ourselves, the clock prepares the body and mind for restful sleep at night and active wakefulness during the day. This clock is referred to as the "circadian system" (Latin: circa dies - about a day) because the cycles it generates have a period length that is not exactly 24 hours, but is faster or slower than that figure. Thus, for humans the figure is about 24.3 - 25.0 hours, depending on the individual.

This means that the circadian system requires time cues or *zeitgebers* (German: time giver) from the environment in order to keep it exactly in tune with the 24 hour rotation of the Earth.

Night workers and people who travel rapidly across time zones run into problems that arise from their circadian systems. Sleep is often interrupted or shortened and daytime mood, alertness and performance impaired. Study of sleep, circadian rhythms, and performance in space allows us to understand what happens to people when they are removed from most of the time cues on Earth. Findings from our experiment will thus help us to understand the actions of *zeitgebers* on the human circadian system, and will help us in providing useful coping strategies to night workers and those suffering from jet-lag.

FY97 Publications, Presentations, and Other Accomplishments:

Carrier, J. and Monk, T.H. "Estimating the endogenous circadian temperature rhythm without keeping people awake." *J. Biol. Rhythms*, vol. 12, 266-277 (1997).

Monk, T.H. and Carrier, J. "Speed of mental processing in the middle of the night." *Sleep*, vol. 20, 399-401 (1997).

Monk, T.H., Buysse, D.J., Billy, B.D., Kennedy, K.S., and Kupfer, D.J. "The effects on human sleep and circadian rhythms of 17 days of continuous bedrest in the absence of daylight." *Sleep* (In Press).

Monk, T.H., Buysse, D.J., Reynolds, C.F., Berga, S.L., Jarret, D.B., Begley, A.E., and Kupfer, D.J. "Circadian rhythms in human performance and mood under constant conditions." *J. Sleep Res.*, vol. 6, 9-18 (1997).

Developmental Analysis of Seeds Grown on Mir

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Funding:

UPN/Project Identification: not available

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 2

FY 1997 Funding: \$75,000

Flight Information:

Experiment ID: 9401653

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Ames Research Center

Task Description:

The developing seeds of *Brassica rapa* provide well-studied systems which can serve as models for comparison of developmental processes in 1-G and microgravity. Embryogenesis and seed formation comprise a complex sequence of cellular interactions and organizational and developmental changes, culminating in a structure whose form and configuration directly reflect these changes. We propose to use these developing seed systems as detectors and amplifiers of space flight effects. *Brassica rapa* will be grown from seed-to-seed for three generations in the Svet hardware on Mir. In-flight gas exchange measurements will test a hypothesis advanced from previous studies on the shuttle, that reproductive development may be limited by reduced photosynthate availability in microgravity. The results will also determine the effects of the spaceflight environment on plant growth and development over multiple generations.

During FY 1997, work focused on refining hardware kits and instructional materials for use by the astronaut on Mir to conduct the experiment, and on tests of a substrate to replace the Balkanine substrate that had previously been used in the Svet greenhouse. The PI attended numerous Science Working Group meetings and performed two crew training sessions at Johnson Space Center. Extensive testing and documentation of all of the kits as well as fixative and substrate were the responsibility of this laboratory. Kit contents prepared by the PI's lab team were assembled at Kennedy Space Center prior to launch of the shuttle to Mir in May. The experiment was initiated when seeds were planted in the Svet greenhouse May 31, and concluded with a final harvest on September 29. Retrieval and processing of the material occurs in the next fiscal year.

Long-term experiments in space on multiple generations of plants have been a dream for investigators hoping to understand the role gravity has played in shaping plant growth and development here on Earth. By studying plant reproduction in the absence of gravity, we can understand the interaction between the plant and its environment in new ways. This knowledge can then provide insight that will lead to new approaches to old problems that we face here on Earth in the area of plant productivity. This particular experiment is unique in

this category because it examines space flight effects on growth and reproduction of plants over multiple generations and thus will be our first opportunity to view these processes over an extended time scale.

FY97 Publications, Presentations, and Other Accomplishments:

Musgrave, M.D., Kuong, A., and Matthews, S.W. "Plant reproduction during spaceflight." *Planta*, 203, S177-S184 (1997).

Musgrave, M.E. "Space can't wait." *Nature*, 383, 823 (1997).

Musgrave, M.E. "Plant reproduction in spaceflight environments." International Space Station Symposium, American Society for Gravitational and Space Biology Annual Meeting, Charlotte, NC (October, 1996).

Musgrave, M.E. "Plant reproduction under spaceflight conditions." Seminar at the NASA Specialized Center for Research and Training in Gravitational Biology, North Carolina State University, Raleigh, NC (July, 1997).

Musgrave, M.E., Kuany, A., and Porterfield, D.M. "Plant reproduction in spaceflight environments." *ASGSB Bull.*, 10, 83-90 (1997).

Xiao, Y., Kuang, A., Porterfield, D.M., and Musgrave, M.E. "Substrates for growth of *Brassica rapa* in the PGF and SVET." *ASGSB Bull.*, 10, 40 (1996).

Analysis of Volatile Organic Compounds on Mir Station

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1995
Students Funded Under Research: 6
FY 1997 Funding: \$ 144,000

Solicitation: 94-OLMSA-01
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9401666
Flight Assignment: NASA 4/Mir 23, NASA 5/Mir 24
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: SSAS, GSC

Task Description:

The goal of this research is the characterization of volatile organic compounds (VOCs) in air samples from the Mir Space Station using new technology based on ion trap mass spectrometry. Twenty-four hour time-averaged samples will be collected onto cartridges using the U.S. Solid Sorbent Air Samples. Grab samples will be collected using U.S. Grab Sample Containers. Samples will be transferred from Mir via the Space Shuttle, forwarded to the Toxicology Laboratory at the NASA Johnson Space Center (JSC) for analysis and sample subdivision, and then sent on to San Francisco State University for the purposes of this work. Standard operating procedures, quality control samples, and confirmatory experiments will be employed to ensure reliable, high-quality data. Analysis will be performed using both a modified form of EPA-approved gas chromatography/mass spectrometry (GC/MS) methods and new techniques based on direct sampling ion trap mass spectrometry (DSITMS). Significant effort will be put into developing, testing, and demonstrating DSITMS techniques with the requisite sensitivity, selectivity, and speed for real-time monitoring of trace-level contaminants in air. The results of this research will provide detailed information on the types and concentrations of VOCs in the Mir environment. Moreover, the demonstration of new technology and comparison against proven methods will yield valuable information on the feasibility of its use for monitoring air quality in advanced life support systems.

The two major goals of this research are the analysis of VOCs in air samples from the Mir Station using a modified form of EPA-approved GC/MS methods, and the development and testing of new DSITMS methods. Major progress was made towards both of these goals in FY97.

Air samples from the Mir 21 and 22 missions were analyzed via GC/MS. The results of this work were documented in numerous reports for these missions that were prepared and submitted to NASA. Mir 21 samples were analyzed via GC/MS in laboratories at San Francisco State University. Mir 22 samples were analyzed using new, state-of-the-art GC/MS system recently installed in laboratories at NASA Ames Research Center.

This system provided a number of improvements and/or refinements on previous GC/MS-based systems for the analysis of trace levels of VOCs in air. The air concentrator exhibited good recoveries for VOCs even when analyzing Mir samples that contained high levels of both water and carbon dioxide. A cryocooled GC oven and 150 m column provided outstanding chromatographic resolution for the wide range of VOCs found in the samples. The ion trap mass spectrometer's inherent sensitivity enabled the use of smaller sample volumes than would normally be required for these types of analyses. Software to automate identification and quantitation of individual VOCs greatly simplified data interpretation. Multipoint calibration curves using internal standards were established for more than 60 VOCs, and other quality assure/quality control procedures were implemented. The VOCs identified in the Mir samples represent fairly common VOC classes including chlorofluorocarbons (CFCs), aromatic hydrocarbons, some oxygenated hydrocarbons, and siloxanes. The highest VOC concentrations were for two fluorocarbons which are most likely used as coolants and refrigerating systems on board Mir. Concentrations for all VOCs identified were well below space maximum allowable concentrations (SMACs). Toxicological interpretation of the data is beyond the scope of both Palmer and Belisle and falls more under the scope of the JSC Toxicology Lab.

Significant effort was put into continued research, development, and testing of new DSITMS techniques for real-time monitoring of VOCs in air. This work included developing and evaluating various sample introduction systems, studying the effects of experimental parameters, developing MS and MS/MS methods for target VOCs of interest, generating gas standards and developing calibration curves, and analyzing Mir samples.

Work continued on sample introduction systems. The most experience to date has been garnered on what is called the Continuous Air Monitor (CAM). This device is simple, easy to use, shows minimal carryover, and has detection limits on the order of 50 parts-per-billion (ppb). Other systems of interest include a valve-based system that does not require the use of helium. Both of these systems are described in a manuscript that was recently submitted for publication. New sample introduction systems based on the use of membranes glow discharge ionization are being evaluated as alternatives and may provide lower detection limits.

Excellent progress was made towards understanding the effects of various experimental parameters on DSITMS experiments. During analysis of air samples, ionization is now known to occur mainly via a charge exchange process with neutral air molecules. Characterization of parameters associated with isolation and fragmentation of ions of interest has been completed. A standard operating procedure for optimizing a scan function for MS/MS experiments was generated and represented the foundation of much of the following work.

DSITMS methods can be based on the use of MS, selected ion monitoring (SIM), or MS/MS modes of operation. MS methods were developed to screen for 34 VOCs commonly found on Mir station, and MS/MS methods were developed to positively identify 20 of these compounds. MS and SIM modes have the lowest detection limits, and are usually employed to screen for the presence of VOCs. In contrast, MS/MS mode has slightly higher detection limits, but provides greater selectivity for the target VOCs of interest. This implies a sensitivity/selectivity tradeoff, in which greater selectivity can be obtained at the expense of sensitivity, and vice versa.

Gas standards were prepared to evaluate various sample introduction systems, experimental parameter effects, and compare and contrast MS, SIM, and MS/MS modes of operation. More importantly, they were used to generate calibration curves generated for 7 VOCs routinely found at relatively high concentrations on Mir Station. Some figures of merit from this work using the CAM sample introduction system in conjunction with DSITMS include detection limits on the order of 50 ppb, reproducibilities of 5% relative standard deviation, response times of less than 1 sec, analysis times on the order of 10 seconds, and linearity over 4 orders of magnitude. Note that these detection limits are several orders of magnitude lower than the SMACs for nearly every VOC of interest. It should also be noted that DSITMS analysis times represent a dramatic improvement upon those normally obtained using GC/MS.

These research efforts recently culminated in the first application of DSITMS to the analysis of air samples from Mir. It should be noted that the inherent nature of Mir air samples presented two major complications to these

analyses – low VOC concentrations and limited sample volumes. Nearly all of the VOCs identified in the Mir air samples are present at concentrations below the 50 ppb DSITMS detection limit. Moreover, these samples are usually repressurized and hence further diluted after analyses by both the JSC Toxicology Lab and the co-investigator. The CAM used in this work consumes air samples at rates of up to 1 L/min. The bulk of this sample is not brought into the mass spectrometer but cycled through an open-split interface back to the atmosphere, thus minimizing carryover and reducing response times. The air samples collected on Mir, however, have a finite volume that is further reduced after pulling off aliquots for GC/MS analyses by both the JSC Toxicology Lab and the co-investigator. The net result here is a relatively small (i.e., typically less than 100 mL) volume remaining for DSITMS analyses. Although these complications limited the scope of the DSITMS analyses, they were nevertheless performed on Mir 22 samples. Results compared well with those from GC/MS analyses, with DSITMS detecting every VOC identified at concentrations greater than 50 ppb via GC/MS. Concentrations from the two different methods in showed good agreement. An interesting sidelight was the detection of sulfur hexafluoride via DSITMS. It should be noted that this compound was not indicated in the co-investigator's GC/MS data. This is understandable given that both cryoconcentration and GC parameters were optimized for VOCs, whereas sulfur hexafluoride is a permanent gas. These results indicate the utility of DSITMS in covering "blind spots" in GC/MS analyses and show its utility for monitoring both VOCs and permanent gases.

Future work will continue to monitor VOCs in samples from later Mir missions via both GC/MS and DSITMS. GC/MS techniques will be modified in an attempt to detect nitrogen and sulfur compounds that may be present in the Mir samples. DSITMS techniques will continue to be refined, improved, and characterized.

The GC/MS results have provided detailed information on the types and concentrations of VOCs in the Mir environment and enable a toxicological assessment of the air quality on board Mir. DSITMS results have shown excellent promise for the potential application of this new technique for on-line, real-time monitoring of trace levels of VOCs in an advanced life support monitoring system. This technique may also find use in numerous Earth-based applications including indoor air quality monitoring, atmospheric monitoring, ecosystems monitoring, stack monitoring, fence-post monitoring, hazardous waste site monitoring, explosives detection, drug detection, and breath analysis.

FY97 Publications, Presentations, and Other Accomplishments:

Coffee, K., Lee, L., and Palmer, P.T. "Direct sampling ion trap mass spectrometry for real-time monitoring of monoterpenes." Western Regional Meeting of the American Chemical Society, San Francisco, CA (October 28 - November 2, 1996).

Fan, X. and Palmer, P.T. "Air quality monitoring via direct sampling ion trap mass spectrometry." 214th ACS National Meeting, Las Vegas, NV (September 7 - 11, 1997).

Fan, X. and Palmer, P.T. "Using MS/MS for environmental analysis - practical considerations." Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA (June 1 - 5, 1997).

Lee, L., Coffee, K., and Palmer, P.T. "Monitoring monoterpenes via tandem mass spectrometry." Western Regional Meeting of the American Chemical Society, San Francisco, CA (October 28 - November, 1996).

Palmer, P.T. "Analysis of volatile organic compounds on Mir Space Station." Society of Western Analytical Professors, University of Arizona, Tucson, AZ (February 14 - 15, 1997).

Remigi, C. and Palmer, P.T. "Analysis of aromatic hydrocarbons in air via direct sampling ion trap mass spectrometry." Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA (June 1 - 5, 1997).

Ziarten, M. and Palmer, P.T. "Monitoring volatile organic compounds in air via ion trap mass spectrometry and expert systems." 213th National ACS Meeting, San Francisco, CA (April 13 - 17, 1997).

Microbial Investigations of the Mir Station and Crew

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Funding:

UPN/Project Identification: 5.1
Initial Funding Date: 1994
Students Funded Under Research: 0
FY 1997 Funding: \$200,000

Solicitation: US/RSA Negotiations
Expiration: 1999
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9400510
Flight Assignment: SLM-1A (Spacelab-Mir)
Responsible NASA Center: Johnson Space Center

Task Description:

Studies will be performed before, during, and after a 90 day mission aboard the Mir space station to characterize the microbial ecology of the crewmembers and space station hardware. Our hypothesis is that qualitative and quantitative changes in human microbiota and in the microbial ecology of the Mir space station will occur due to confinement of the crew in the space station's microgravity and closed system environment. Furthermore, the confinement of crew will allow for alterations in body microflora and transfer of microorganisms among crewmembers directly and through the environment. Air, water, interior surfaces, and crewmember samples will be collected from the Mir, pre-, in-, and postflight and analyzed for their microbiological makeup. Microbial samples will also be taken from the interior surfaces and from the water and air system of the Soyuz spacecraft and Space Shuttle used in support of these missions. Additional water samples will be taken from the Progress spacecraft used to transport supplies to the Mir from Russia. All samples will be qualitatively and quantitatively analyzed for bacteria and fungi. Water samples will also be analyzed for viruses. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida albicans* and other appropriate target microbes isolated from any environmental or clinical sample will be analyzed genetically to associate the microbe with a primary source. Specific goals of this study are: (1) to characterize the microorganisms associated with air, surfaces, water, and crewmembers before, during, and after a 90 day mission to the Mir space station; (2) to determine extent of microbial transfer among crewmembers; and (3) to assess the dissemination of crew microbiota throughout the Mir space station. The overall scope of this study is to describe the microbial colonization of a space station as well as define the impact of environmental microbes on crew health.

Crew Microbiology

Characterization of microbiota has been performed on the Mir 22/NASA 3, NASA 4, and NASA 5/Mir 24 crewmembers. Monitoring was performed preflight and postflight on all crewmembers. Inflight samples were

collected on Mir 22, NASA 3, and NASA 4 crewmembers; samples were frozen and returned to ground for analysis. Specimens included throat, nose, ear, hand, scapula, axilla, groin, urine, and feces cultures (Note: urine and feces not performed inflight). All cultures included quantitation and identification of bacteria and fungi to the species level when possible. Preflight and postflight sampling was performed on the NASA 5 crewmember; inflight sampling was planned but aborted due to the Spektr accident. Compilation of this data has not been completed. Preflight sampling was performed on the Mir 24 and NASA 6 crewmembers.

Environmental Microbiology

Characterization of microbiota from the Mir air, surfaces and water was performed on the Mir 22/NASA 3, and Mir 23/NASA 4 missions. Samples were collected and processed inflight. Incubation and an estimated quantitation were performed; all specimens were returned to ground for identification of bacteria and fungi. During the Shuttle docked phase of these missions, archival samples were collected and returned to ground for quantitation and identification of bacteria and fungi. Air and surface sampling was also performed on the Shuttle prior to docking and during the docked phase with the Mir. These samples were returned to ground for quantitation and identification of bacteria and fungi. Samples collected and planned during the NASA 5 mission were aborted due to the Spektr accident. For the most part, environmental microbial loads have been within the acceptability levels established for the International Space Station. In general, the microbiological profile of the Mir has been comparable to that of the Shuttle and Spacelab.

Accumulating evidence suggests that the human immune response may be attenuated during space flight. To control the development, transmission, and treatment of infectious diseases, the effects of space flight both on microorganisms themselves and on the human immune response must be understood. This study will help in adding to the body of knowledge with regard to the mode of action of microbial infection - a problem that is directly associated with immune compromised individuals on Earth.

Microbes' colonization of inanimate surfaces and hardware of the spacecraft can also lead to biodeterioration of critical life support instrumentation and equipment as well as the release of toxic volatiles. All these are problems associated with an Earth problem commonly called "sick building syndrome" (SBS) or "building-related illnesses." Reducing risk to SBS requires monitoring both the habitation environment and the occupants, such that the levels and types of microbes do not reach critical levels. A thorough understanding of the microbial population dynamics on board spacecraft will allow for development of predictive measures that can be used on Earth. The information gained from this study will be helpful in the design of future spacecraft as well as environmentally conscience buildings, and development of monitoring requirements in order to minimize microbial cross-contamination.

FY97 Publications, Presentations, and Other Accomplishments:

Isenberg, H.D., Novikova, N.D., Lizko, N.N., Viktorov, A.N., Mishra, S.K., Groves, T.O., and Pierson, D.L. "Effects of long-duration space missions on crew body microflora." (Poster) Annual Meeting of the American Society for Microbiology (May, 1997).

Koenig, D.W., Bell-Robinson, D.M., Groves, T.O., Barta, D.J., and Pierson, D.L. "Effects of human habitation on the microbiology of an advanced life support chamber." (Poster) 97th General Meeting, American Society for Microbiology, Miami Beach, FL (May 4 - 8, 1997).

Koenig, D.W., Bruce, J.L., Bell-Robinson, D.M., Ecret, L.D., Valadez, V.A., Zakaria, Z., and Pierson, D.L. "Analysis of bacteria isolated from water transferred from the Space Shuttle to the Mir Space Station." (Poster) 97th General Meeting, American Society for Microbiology, Miami Beach, FL (May 4 - 8, 1997).

Mehta, S.K., Grimm, E.A., Smid, C., Kaur, I., and Pierson, D.L. "Decreases in NK-cell cytotoxicity after space flight." (Poster) 97th General Meeting, American Society for Microbiology, Miami Beach, FL (May 4 - 8, 1997).

Pierson, D.L., Mehta, S.K., Brauning, C., and Winestock, G. "DNA fingerprinting - an application to the space microbiology." (Poster) 12th Man in Space Symposium, Washington, DC (June 8 - 13, 1997).

Pierson, D.L., Viktorov, A.N., Lizko, N.N., Novikova, N.D., Skuratov, V., Groves, T.O., Bruce, R.J., Mishra, S.K., and Koenig, D.W. "Microbiology of the Mir Space Station and flight crew during the Mir-19 mission." (Poster) Annual Meeting of the American Society for Microbiology, Miami, FL (May, 1997).

Viral Reactivation

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Funding:

UPN/Project Identification: 2.4.3

Solicitation: US/RSA Negotiations

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Flight Information:

Experiment ID: 9400243

Flight Assignment: SLM-1A (Spacelab-Mir)

Responsible NASA Center: Johnson Space Center

Task Description:

Several strains of herpes virus are commonly found in humans. These viruses cause cold sores and other infections. Once a person is infected with the virus, it may be present for life and can be reactivated by several factors, including stress. Scientists believe that the stresses associated with space flight may increase the incidence of reactivation of latent herpes virus in crew members during a long-duration mission.

This study investigates the influence of space flight upon the frequency and magnitude of reactivation and shedding of clinically important latent viruses in saliva. Saliva samples were collected from the three Mir 18 prime and three backup crew members during a two-month preflight period to establish baseline values. The objective of this experiment is to detect and identify any reactivated herpes viruses in saliva specimens collected from the subjects before, during, and after their stay on Mir. Saliva samples are collected and examined for the presence of activated viruses using Polymerase Chain Reaction (PCR) technology. This technology is the latest, most up-to-date procedure for conducting DNA analysis.

This project was terminated with the Mir 19 mission.

The rapid and accurate diagnosis of herpes virus infections is extremely important. Herpes virus infections (e.g., Herpes simplex encephalitis) is severe and in many cases is fatal without treatment. This research has resulted in advanced methods of detection using PCR methodology. This advanced technology has resulted in application of a Technology Transfer. Additionally, a highly sensitive set of Cytomegalovirus (CMV) primers was developed for this application (and is the subject of a U.S. patent-JSC/AL3), and a collaborative study is being set up with Baylor College of Medicine to use these primers for detecting CMV DNA in patients at Texas Children's Hospital. Benefits of this technology include rapid identification of herpes virus infections, allowing treatment to stop the spread of infection.

FY97 Publications, Presentations, and Other Accomplishments:

Pierson, D.L., Mehta, S.K., Brauning, C., and Weinstock, G.M. "DNA fingerprinting: Application to space microbiology." 12th Man in Space Symposium, The Future of Humans in Space, Washington, DC (June 8 - 13, 1997).

Assessment of Humoral Immune Function During Long Duration Space Flight

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Irina Rykoua, Ph.D.; Institute for Biomedical Problems, Russia

Funding:

UPN/Project Identification: E621

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$99,000

Flight Information:

Experiment ID: 9401621

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

The changes in immune function which occur during space flight potentially expose the crews to an increased risk for development of illness. Decreased cellular immune function has been repeatedly documented after space flight and confirmed during flight by *in vivo* delayed-type hypersensitivity testing. The mechanisms of these responses and the involvement of the different arms of the immune system are currently unclear. Our hypothesis is that space flight will cause a decrease in humoral immune function similar to that observed with the cell-mediated immune system. To test this hypothesis, crew member volunteers will be immunized with polysaccharide antigens and the production of immunization specific antibodies will be determined. The immune responses generated during flight will be compared to responses from a synchronous ground-based control group. Assessment of *in vitro* B cell function will also be performed. A thorough understanding of the immune system function during space flight is critical to the assessment of crew health risks.

An in-flight vaccination with specific antigens (23 pneumococcal polysaccharide antigens) were used to test the ability to mount an antibody response *in vivo*. This vaccination was performed on two NASA/Mir flight increments in FY97. Blood and saliva samples were collected prior to flight and immediately before immunization in-flight to determine baseline values of the specific antibodies. Vaccination took place approximately mid-mission. Additional saliva and serum samples were taken 7, 11, 14, 17, 21, and 28 days after the immunization to measure the degree of antibody production. Antibodies to the immunization antigens will be measured by enzyme-linked immunosorbent assay (ELISA) in the serum and saliva samples.

An initial measurement of the pre-immunization antibody titers for the preflight and inflight samples has been performed for the 4 most common pneumococcal isotypes. Analysis of the remaining samples will be

performed following collection of the remaining flight samples. The samples will be analyzed in batch and with their age/sex matched ground controls. The degree of response will be compared to those obtained from a ground-based control group of age- and sex-matched volunteer subjects.

The focus of this experiment is to understand the effects of space flight on crew member immune function, and the results have their major relevance in this arena. However, if differences are found, elucidation of the factors mediating this response will provide new insight into the maintenance of human immune function in health and disease.

Humoral Immunity

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Funding:

UPN/Project Identification: 2.4.2
Initial Funding Date: not available
Students Funded Under Research: 0
FY 1997 Funding: not available

Solicitation: US/RSA Negotiations
Expiration: not available
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9400242
Flight Assignment: SLM-1A (Spacelab-Mir)
Responsible NASA Center: Johnson Space Center

Task Description:

The immune system has two basic components which mediate specific immune responses: the humoral and cell-mediated immune systems. The humoral component involves the production and action of lymphoid products called antibodies. The cell-mediated component encompasses functions directly performed by sensitized lymphocytes. Investigators believe that the immune system is affected by the changes in the body that occur in space. It has been proposed that the ability to mount an antibody response to foreign substances (called antigens) is reduced during space flight, and that the concentration of specific antibodies following immunization during flight will be significantly lower than responses obtained from a ground-based control group. The objective of this experiment is to determine whether the humoral component of the immune system is capable of mounting a response to an antigen during space flight. Blood and saliva samples are collected before crewmember subjects receive a vaccination, then at 1, 2, and 3 weeks after the vaccine. Subjects will be vaccinated during SL-M and samples collected post flight. The levels of antibodies produced by the body are measured in serum and saliva samples.

Postflight analysis of the samples is essentially complete. Analysis of the preflight, inflight, and postflight antibody levels in the serum samples has been completed for the four primary isotypes (type 3, type 7F, type 9N, and type 14). The samples were also analyzed for the levels of serum immunoglobulins (IgG, IgA, IgD, IgE, and IgM). While the data are limited, some trends are apparent. The three crewmembers all exhibited minor shifts in the levels of isotype specific antibodies between the preflight baseline and the inflight baseline. In general, these do not appear to be highly significant, and they suggest the serum antibody levels are not altered directly by space flight. The immunized crewmembers did respond to the vaccine by increasing antigen-specific antibody titers. The initial analysis of the antibody levels indicates the crewmembers were able to mount a response to the immunization during flight. However, the degree of response may be altered for

some isotypes in some individuals. General immunoglobulin levels did not change significantly during or after flight in any of the crewmembers. This was consistent with previous observations.

The data from the Mir 18/STS 71 constitute the first part of this investigation. Further subjects will be obtained during the course of the Phase 1B science program on Mir.

The focus of this experiment is to understand the effects of space flight on crewmember immune function, and the results have their major relevance in this arena. However, if differences are found, elucidation of the factors mediating this response will provide new insight into the maintenance of human immune function in health and disease.

Peripheral Mononuclear Cells

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Funding:

UPN/Project Identification: 2.4.4
Initial Funding Date: not available
Students Funded Under Research: 0
FY 1997 Funding: not available

Solicitation: US/RSA Negotiations
Expiration: not available
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9400244
Flight Assignment: SLM-1A (Spacelab-Mir)
Responsible NASA Center: Johnson Space Center

Task Description:

This investigation focuses on the cellular branch of the immune system. Previous research has suggested that extended exposure to microgravity results in altered characteristics of immune cells. The objective of this experiment is to understand the effects of space flight on the human immune system by determining the effects of space flight on circulating immune cells.

To begin this experiment, blood samples are collected from crewmembers. From these samples, investigators isolate white blood cells, stain them for specific markers, and analyze them using flow cytometry. Analyses to determine the functional competence of the monocytes, natural killer cells, and T cells are also performed. This experiment is conducted preflight, on the Shuttle just prior to landing, and postflight.

Preliminary Science Findings:

The circulating subpopulations of peripheral white cells were not greatly altered during flight as compared to the preflight baselines. In contrast, the samples taken within 2-3 hours after landing had the relative granulocytosis and lymphopenia observed after most space flights. A relative decrease in T cells, monocytes, B cells, and NK cells was also noted after flight. These changes were not observed in the inflight sample taken during the last flight day, suggesting that they are an acute response to reentry and readaptation to unit gravity.

In vitro activation of lymphocytes isolated from crewmembers during flight or immediately after flight was not significantly different from the control periods as determined by the expression of early activation markers. Expression of CD 69 protein or IL-2 receptor (CD 25) 24 hours after mitogen activation was not altered relative to preflight control samples. All crew members exhibited good NK cell function on landing day; however,

variable decreases in cytotoxicity were noted 9 days after landing. The reasons for this effect are currently unclear, though it is consistent with previous observations during long-duration space flight on Mir.

Conclusions:

Statistical significance is limited by the low sample number of the study; however, some interesting trends are suggested by the current results. It appears that the major phenotypic changes which are observed after landing arise as a result of reentry and reambulation. Another interesting observation was that expression of early activation markers in cells activated *in vitro* immediately after flight was not substantially different from the preflight values. This differs from previous observations which show a decrease in lymphocyte proliferation after flight. These data suggest a decoupling between early activation events and the control of cell cycle progression/proliferation in the T cells. This could have significant impact on immune regulation, as these functions are closely regulated during the coordination of an immune response. Further research will be required to determine the importance of these findings.

This task is focused on the examination of the effects of space flight on human immune cells. This investigation will, however, provide insight into mechanisms which regulate human immune function. These studies will improve the understanding of the effects of psychological and physical stress on specific components of the cellular immune system. The examination of specific cell functions and changes in the cytokine patterns should be particularly relevant to the regulation of immune responses in health and disease.

Collecting Mir Source & Reclaimed Waters for Postflight Analysis

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Funding:

UPN/Project Identification: E592

Solicitation: 94-OLMSA-01

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$199,000

Flight Information:

Experiment ID: 9401592

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

Reclamation and purification of waste waters, as is currently done on the Russian Space Station Mir, will be required for supplying crewmembers of the International Space Station with potable and hygiene water. Contaminants released through metabolic functions of humans, off-gassing of hardware, and flight experiments and operations will be present in spacecraft waste waters. To ensure that crew health is maintained during extended missions, all water intended for human use must meet established water quality standards. This investigation will provide critical information on specific contaminants in Mir waste water and reclaimed water. The objectives of this experiment are to determine the potability of the water supplied on Mir, to assess the reliability of the Mir potable water systems, and to aid in developing water quality monitoring standards for International Space Station. Results of the analysis of water samples collected during the Mir 18-Mir 23 missions show that only up to 11% of the constituents of the reclaimed water could be identified using present analytical techniques. Results show the reclaimed water met all requirements of the Joint U.S./Russian spacecraft water quality standards except for total organic carbon and turbidity.

During FY 97, potable water and condensate samples were analyzed from the Mir 22 and Mir 23 missions. Eleven samples were collected and analyzed from Mir 22 and nine samples were analyzed from the Mir 23 mission. During these missions, leaks from the thermal control system in the Mir core, the Kvant, and the Kvant II modules of the Mir Space Station required shutting down the Condensate Recovery System (CRS) which reclaims potable water from humidity condensate because of possible contamination of the potable water by the thermal coolant, 1,2-ethanediol (ethylene glycol).

Accomplishments during FY97 include the improvement of some analytical methods for the postflight analysis of the Mir water samples and the development of a preliminary database on the quality and composition of

spacecraft waters. In addition, critical data was provided to respond to the ethylene glycol leaks onboard the Mir. New hardware has subsequently been developed to ensure that the CRS could adequately remove large concentrations of ethylene glycol.

This research will provide benefits in the areas of methods development for the analysis of drinking water, advanced technologies for the treatment of wastewaters, and increased knowledge of potable water contaminants. Improvements in methods development as a result of this experiment will potentially increase the sensitivity of organic analyses 10-fold over present techniques. These improvements will allow more complete characterization of potable water, accounting for most organic constituents even those at extremely low levels. In addition, by adapting techniques for treating spacecraft waters, the development of better wastewater treatment technologies on Earth will be supported.

FY97 Publications, Presentations, and Other Accomplishments:

Pierre, L.M., Schultz, J.R., Sauer, R.L., Sinyak, Y.E., Skuratov, V.M., and Protasov, N.N. "Chemical analysis of potable water and humidity condensate collected during the Mir-21 mission." 27th International Conference on Environmental Systems, Lake Tahoe, NV (July 14 - 17, 1997).

Bone Mineral Loss and Recovery after Shuttle/Mir Flights

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Funding:

UPN/Project Identification: not available

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$640,000

Flight Information:

Experiment ID: 9401598

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

Our research group has participated in a joint Russian/American research project to determine the bone mineral loss of cosmonauts after long-duration space flight lasting from 4 to 14 months. This program was the first to study bone loss in weightlessness in a comprehensive manner and included measurements of the spine, hip, tibia, whole body, and subregions of the whole body. To date, 18 cosmonauts have been studied. While this study is extremely valuable, there is only limited data on the very important issue of recovery of bone after return to 1-G. Knowledge of the rate and degree of bone recovery is important not only for NASA, but for clinical investigators interested in reversing the effects of osteoporosis. This proposal will measure the space flight-induced bone loss of the twelve crew members of the Shuttle/Mir flights and follow the recovery with bone mineral measurements every six to twelve months for up to three years or until full recovery has occurred. In order to gain information on the role of muscular fitness with respect to bone loss and recovery, muscle strength testing will be performed at the same time points as bone mineral measurements. Muscular fitness will be used as an indicator of a crew member's level of load-bearing physical activity throughout the study. Serum and urinary markers of bone metabolism will be measured pre- and post-flight in order to provide information regarding the altered metabolism of bone resulting from long-duration flight. This information will complement the bone density results and may shed light on the mechanisms involved in disuse bone loss and subsequent recovery.

During 1997, the investigators completed DEXA and Lido data collection to include 6- and 12-month recovery data on NASA-2; landing, 2- and 6-month recovery data on NASA-3; landing and 6-month recovery data on NASA-4; preflight and landing data on NASA-5; and preflight data on NASA-6. Pre- and postflight data were collected on the Mir crews. Mir-21 and -22 recovery data are behind schedule with the first recovery 6-month and 1-year data in the process of collection at this time. No correlation of recovery strength and bone density recovery can be made at this time. One NASA crew member has shown complete recovery at 6 months. All other NASA crewmembers still show significant losses with incomplete recovery.

Results from this study should provide insight into the role of decreased physical activity in the development and treatment of osteoporosis—a costly and debilitating condition which affects millions worldwide. Recovery data obtained during the 3-year post-flight period should provide valuable information regarding the rate and extent of bone recovery following disuse. Muscle mass and strength data may provide additional insight into the role that muscle fitness plays in bone loss and, particularly, bone recovery. Knowledge of the rate and degree of bone recovery is important not only for NASA, but for clinical investigators interested in reversing the effects of osteoporosis. Knowledge of the sensitivity of serum and urinary markers of bone metabolism to track bone loss and recovery will provide a clearer understanding of the usefulness of these markers to monitor alterations in bone metabolism and may shed light on the basic biological mechanisms involved in bone loss and recovery.

FY97 Publications, Presentations, and Other Accomplishments:

Shackelford, L., Feiveson, A., Spector, E., LeBlanc, A., and Oganov, V. "Prediction of femoral neck bone mineral density change in space." 12th Man in Space Symposium, Washington, DC (June, 1997).

Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1995
Students Funded Under Research: 2
FY 1997 Funding: \$10,000

Solicitation: 93-OLMSA-06
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9306016
Flight Assignment: Euro-Mir
Responsible NASA Center: Ames Research Center

Task Description:

The purpose of this research is to investigate the fundamental effects of gravity deprivation on the visuo-vestibular system of birds. In particular, the distributions of various neurochemicals during development are being analyzed by using immunohistochemical techniques. Development of the visual brain is also being studied by measuring the volumes of the optic tectum.

Visual information plays an important role for the vestibular functions. In normal settings, movements of the visual field or the body induce compensatory movements of the eyes and/or the head in order to stabilize the retinal image. In the situation of no gravity, however, the visual motion is irrelevant information to the vestibular functions. Little is known about the influence of gravity on the neural development of the visuo-vestibular system. Is gravity a critical stimulus for the normal development of the neural structures of the system? There are at least five major structures involved in the visuo-vestibular interactions: 1) optic tectum, 2) accessory optic system, 3) pretectum, 4) vestibular nuclei, and 5) vestibulo-cerebellum. Previous studies suggest that at least the following neurochemicals exist in the visuo-vestibular structures: two types of neurotransmitters—serotonin and gamma-aminobutyric acid; three types of enzymes—tyrosine hydroxylase, dopamine beta hydroxylase, and choline acetyltransferase; three types of peptides—substance P, neuropeptide Y, and cholecystokinin; and two types of calcium binding proteins—parvalbumin and calbindin. In the proposed study, the distributions of these 10 neurochemicals in the five visuo-vestibular structures are being studied in quail.

Tissue Collection: Brain samples include the forebrains (telencephalon, diencephalon) and a part of the mesencephalon (optic tectum) collected by dissection at Ames Research Center in October, 1996. Ten brain tissue samples have been received from the lab control groups (6-E16s and 4-E14s), seven from the synchronous

controls (4-E16s and 3-E14s), and four from the flight groups (2-E16s and 2-E14s). Among the tissues, some were not well fixed and thus could not be processed for a histochemical analysis. Tissues which were well fixed have been analyzed histochemically. Additional synchronous and lab controls were also obtained by dissection at Ames in June, 1997. The data from the new controls are not included in this report. Furthermore, Dr. Dmitri Lytchakov (Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences) worked in our lab for two weeks in July, 1997, to collaborate and analyze his tissue samples which were also obtained by the dissection at Ames in 1996. The data analysis from this collaboration has not yet been completed and thus is not included in this report.

Tissue Preparation: The basic procedures have been described elsewhere (Shimizu & Karten, 1990). Briefly: tissues were received in a fixative of 4% paraformaldehyde in phosphate buffer (PB). The tissues were post-fixed in a mixture of 4% paraformaldehyde and 30% sucrose-PB, and stored at 4 degrees C for at least 12-16 hours. They were then sectioned 30-35 μm thick on a cryostat in sagittal stereotaxic planes, and mounted on gelatin-coated glass slides. Sections on the slides were then washed three times in PB at room temperature for at least 30 minutes, and then incubated in the primary antisera or antibodies diluted in PB with 0.3% Triton X-100 at 4 degrees C for 15-20 hours.

Antibodies: Four types of antibodies against calcium-binding proteins (CaBPs) and an enzyme were used for staining. They were 1) anti-calbindin-D (CaBP; Sigma, #C-8666, monoclonal mouse, 1:1,000 dilution); 2) anti-calretinin (CR, Chemicon, #AB149, polyclonal rabbit, 1:1,000 dilution); 3) anti-parvalbumin (PV, Sigma, #P-3171, monoclonal mouse, 1:1,000 dilution); and 4) anti-tyrosine hydroxylase (TH, Incstar, #105440, monoclonal mouse, 1:1,000 dilution). All four antibodies are commercially available. Preliminary studies with pigeons and finches show that these antibodies provide good staining in the avian forebrain.

Immunohistochemistry: Following the primary incubation, sections were washed three times with PB for at least 30 minutes and processed according to the avidin-biotin (ABC) method. The sections were incubated in various biotinylated secondary antibodies. The incubation was carried out at a dilution of 1:100 in 0.3% triton X-100 in PB at room temperature for one hour. The tissues were then washed three times for 30 minutes, drained, and incubated in avidin-coupled peroxidase (Vector ABC kit) at a dilution of 1:100 in 0.3% triton X-100 in PB at room temperature for one hour. After the washes in PB, the sections were incubated with 0.025% 3,3'-diaminobenzidine (Sigma) in PB for 15 minutes. Hydrogen peroxide was added to the medium to a final concentration of 0.01%. The reaction continued for 15-20 minutes. Sections were then washed several times in PB, then air-dried. The sections were dehydrated and coverslipped with Permount. Control conditions for immunohistochemistry were carried out but with the omission of the primary antibody incubation.

Additional Staining and Data Analysis: Adjacent sections were Nissl-stained with cresyl violet (Pfalts and Bauer) in order to 1) identify boundaries and 2) study the size and density of neurons in different cell groups. Sections were examined with dark-field, standard bright-field, and Nomarski microscopy. Charting was carried out using a camera lucida and/or an image analysis program (Image in Macintosh computer) through a video image.

Results:

1) **Immunohistochemistry:** With samples where fixation was adequate, the tissues were well stained with all three antibodies against CaBPs and TH, as well as Nissl staining. However, samples with incomplete fixation, including all flight cases, showed staining only for CB, but not for CR, PV, and TH. Positive staining for CB was already visible in the E14s. The CB-positive areas include the tectal layers, thalamic nuclei (e.g., nucleus rotundus, hypothalamus), sensory telencephalic areas (e.g., nucleus basalis, ectostriatum), and hippocampal complex. The intensity of the staining varied considerably within each group (i.e., lab, synchronous, and flight) probably due to the difference in the degree and extent of the fixation. No significant differences in staining patterns, however, were observed among the different groups.

2) **Volume Measurement of the Optic Tectum:** The avian optic tectum is the major retinorecipient structure with well-developed laminations. When fixation was adequate, these layers were clearly stained with CB and

Nissl stainings. Part of the optic tectum of some samples were either damaged or detached due to an incomplete fixation, and thus no accurate measurement was possible for these samples. Accordingly, no samples from the flight group were used for this analysis. In the lab control group, the estimated volumes of the optic tectum are 15.41mm^3 for E16s, and 13.36mm^3 for E14s ($n = 10$). In the synchronous group, the volumes are 13.23mm^3 for E16s, and 10.15mm^3 for E14s ($n = 3$). Although these figures suggest that the tecta of the synchronous group are much smaller than those of the lab control group, the sample size was so small that no statistical comparison is possible. As for the flight group, we are currently measuring volumes of more rostral brain structures (e.g., the basal ganglia) which are involved in sensory and motor functions. These structures were undamaged with an incomplete fixation.

Conclusions:

1) Neurons of the selected brain regions express CB by E14. Since no systematic differences in the staining patterns have been seen in different groups, the data indicate that the different conditions did not significantly affect the development of these neurons in the brain structures. Further analysis will be conducted to examine the detailed morphology and distribution of these cells to confirm this conclusion.

2) Although the optic tecta of the lab group appear to be larger than those of the synchronous group, the sample size was too small to carry out a meaningful comparison. Furthermore, the data of the flight group was not included in the analysis. However, the additional control samples obtained from the latest dissection (June 1997) will allow us to increase the sample size. Moreover, the size and volume of non-tectal brain structures will be measured in the subsequent analysis in order for the data from the flight group to be included.

Visual information plays an important role for the vestibular functions. In normal settings, movements of the visual field or the body induce compensatory movements of the eyes and/or the head in order to stabilize the retinal image. In the situation of no gravity, however, the visual motion is irrelevant information to the vestibular functions. Little is known about the influence of gravity on the neural development of the visuo-vestibular system. Is gravity a critical stimulus for the normal development of the neural structures of the system? Are particular cell groups more susceptible to gravity deprivation? The present study is evaluating the effects of gravity deprivation on the visuo-vestibular system. In particular, the neurochemical nature of the system is being explored using immunohistochemical techniques. Such information will be important for understanding the influence of gravity on the development of the central nervous system in general.

FY97 Publications, Presentations, and Other Accomplishments:

Shimizu, T. Effects of weightlessness on the avian visuo-vestibular system: Immunohistochemical analysis. NASA Tech Brief, 4751, 81-82 (1996).

Shimizu, T. Effects of weightlessness on the avian visuo-vestibular system: Immunohistochemical analysis. NASA Tech Brief, 66-67 (1997).

Evaluation of Skeletal Muscle Performance and Characteristics

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1994
Students Funded Under Research: 0
FY 1997 Funding: \$ 108,000

Solicitation: 94 OLMSA-01
Expiration: 1998
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9400411
Flight Assignment: NASA-Mir-1B, Mir-22/NASA-3
Responsible NASA Center: Johnson Space Center

Task Description:

Muscles that are not used lose their strength. In addition to the loss in muscle mass during and after space flight, there is a loss of muscular fitness. This response is similar to observations with prolonged immobilization, such as being bedridden. When reduced fitness occurs, decreases in strength, endurance, tone, and efficiency result. Investigators for this experiment hypothesize that being in a weightless environment results in non-uniform changes (e.g., extensors > flexors, legs > arms) during flight with a slow readaptation to preflight levels upon return to Earth.

One objective of this experiment is the evaluation of how skeletal muscle performance and characteristics adapt during long duration space flight. Investigators then compare post-flight response with preflight values to determine how long it takes (and what mechanisms are used) to readapt to Earth's gravity. The tests protocols included: (1) muscle strength, endurance and tone, (2) neuromuscular efficiency, (3) voluntary and evoked contractions, and (4) integrated muscle performance testing on a passive treadmill. These protocols were performed before and after Mir-18 and helped evaluate the efficacy of the Russian Countermeasures. Evaluating the metabolic cost of passive running on the treadmill during STS-71 helped determine the extent of the postflight change in performance.

Deconditioning of skeletal muscle due to inactivity has its etiology in neural, biochemical, and morphological characteristics. This experiment will focus on the change in skeletal muscle performance and its neural

components. This experiment will also evaluate the efficacy of the Russian countermeasure program on skeletal muscle performance. These will result in a better understanding of muscle function, deconditioning and rehabilitation, and measuring the efficacy of the Russian countermeasure program and its possible use in rehabilitative medicine (physical therapy).

Protein Metabolism During Long Term Space Flights

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: E613	Solicitation: 94-OLMSA-01
Initial Funding Date: not available	Expiration: 1998
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$244,000	

Flight Information:

Experiment ID: 9401613
 Flight Assignment: NASA-Mir-1B
 Responsible NASA Center: Johnson Space Center

Task Description:

Human space flight is associated with a loss of body protein. Specific changes include a loss of lean body mass and decreased muscle strength. The major site of the protein loss is believed to be from muscles which have anti-gravity functions. The weakness of astronauts after long-term space flight has been attributed to this loss of body protein. We have previously shown that short-duration space flight on the Space Shuttle is associated with decreased nitrogen balance, a stress response, the development of insulin resistance, and a transient increase in pro-inflammatory cytokine activity.

The objectives of this experiment are: (i) to determine the duration of the metabolic stress response associated with space flight; (ii) determine the magnitude of the decrease in the whole body protein synthesis rate after the initial adjustment period is over; and (iii) determine how long it takes for protein metabolism to return to its preflight state after a long duration mission. The hypotheses to be tested are: (i) space flight is associated with an initial metabolic stress response which is over sometime within the first few weeks of space flight and then the whole body protein synthesis will decrease; and (ii) after landing, return to the preflight protein metabolism status takes several weeks. We plan to accomplish these goals by measuring the whole body protein synthesis rate, four times before, serially every three to four weeks during space flight and then on four times post flight spaced out between R+1 and R+60. The ¹⁵N glycine method will be used to determine the protein synthesis rates. The preflight measurements are to obtain a baseline, the inflight measurements are to document how long into the flight the whole body protein synthesis rate stays elevated and the magnitude of the eventual decrease, and the postflight measurements are to determine how long it takes for the whole body protein synthesis rate to return to the preflight baseline.

We now have preliminary data on six subjects. At present the database consists of the preflight and inflight whole body protein turnover data. The ¹⁵N glycine method for the measurement of the whole body protein synthesis and breakdown rates (turnover) gives two estimates of the whole body protein synthesis rate, one value is based on the excretion of isotope in the urinary ammonia, the other on the excretion of ¹⁵N in urea. The two

methods complement each other and give similar values. At present only the ammonia-based data is available. The urea-based data analyses are as yet incomplete.

Even though the data is preliminary and partial, the following conclusions can be drawn. After 3 plus months in space, the whole body protein synthesis rate is reduced by about 46% ($p < 0.01$). This is greater than that found with bed rest. Although not presented here in detail, the preliminary analysis of the postflight data shows, as predicted, considerable scatter in the data with no consistent pattern. No comment will be made on this data until we have seen the dietary intake data for that period.

The question of whether humans can truly adapt is of both practical importance and of general biological interest. If a 'mild' but chronic stress response continues with its associated energy and protein wasting, long-term space missions to destinations such as Mars become very problematic unless effective countermeasures are developed. If the stress response is short and finite indicating true adjustment to the new environment, then the problem is known, is limited in duration, and is not serious. Space flight confronts humans with a totally novel situation. Is there enough flexibility in the genetically determined response to stress that humans can adjust to stresses for which there can be no pre-programmed specific response?

Microbial Interaction in the Mir Space Station Environment

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: E703

Solicitation: 94-OLMSA-01

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$165,000

Flight Information:

Experiment ID: 9401703

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

The long-term goal of this project is to understand the nature of microbial behavior in closed, long-duration space flight systems. This will be accomplished in part by applying techniques of molecular genetics. These studies in conjunction with other approaches should provide the information and methodology required to (1) set standards for microbial exposure, (2) develop in flight assays for microorganisms that determine if conditions are within prescribed standards, and (3) implement appropriate procedures to control microbial concentrations when needed.

The hypothesis is that microorganisms have been introduced into the Mir space station over the ten years it has been in orbit from the natural microbial flora of crew members as well as payloads and other on-board sources. These microbes have been dispersed throughout the space station and have settled into specific environmental niches. A unique natural selection for microbes that can flourish in the Mir environment has occurred during this time. Accurate assessment of this unique microbial ecology is critical for understanding the behavior of microorganisms that will inevitably be introduced into any closed-space environment. To this end, we will perform a study using restriction endonuclease digestion and pulsed field electrophoresis to fingerprint microorganisms from space flight missions. These samples will also be used to develop a faster assay based on the polymerase chain reaction (PCR). These techniques will allow microbes introduced by the crew to be distinguished from those already present on Mir. It will also be possible to tell if crew members pick up microbes from the space station environment and whether crew members transfer microbes themselves. Microorganisms that are identified by this procedure as being endogenous to the space station will then be assayed for various properties such as antibiotic resistance, adhesion to surfaces, DNA repair capacity, stress responses, growth in poor media, and secreted proteins and compared to standard strains. This will determine if any special properties are selected for among microbes that persist in the space station.

We have developed a more rapid DNA fingerprinting procedure than previously used. Using 112 strains of *Staphylococcus aureus* (5 from space shuttle missions, 90 strains from Mir 18 and Mir 19, 15 strains of clinical

isolates from local hospitals, and two well-characterized laboratory strains), we ascertained conditions for PCR reactions using whole genome DNA as template and primers based on known bacterial repeat sequences (either the REP or BoxA sequence). We were able to discriminate nearly all strains using this PCR procedure. In the few examples where PCR failed to distinguish strains, further examination by pulsed field gel electrophoresis showed the strains to be nearly identical, differing usually in only one restriction fragment. These small differences would not be sufficient to conclude that the strains were unrelated, so we conclude the PCR method is nearly as sensitive as older procedures, but is much faster and cheaper.

Analysis of the crew samples showed clear examples of microbial transfer among crew during the long duration training period, and persisting through the end of the mission. Some crew members shed high levels of *Staphylococcus aureus* and this was picked up by other crew members (who were otherwise free of this microbe) during training. Notably, some crew members were not colonized by *S. aureus* even though they trained with *S. aureus* carriers and other members who were colonized. There were no acknowledged clinical signs of infection during this period.

Based on the successful application of this technology to *S. aureus*, we are beginning to apply this procedure to the study of other microorganisms. We have analyzed the microbial flora recovered from crew and environmental samples from Mir 18, Mir 19, and Mir 22. This compilation was then sorted into a number of groups. Our initial focus is on those organisms commonly shared among crew (10 different genera comprising many species) and those found post-flight, particularly those that were candidates for transfer either between crew or from the space station to crew (7 different genera). We are now using our PCR procedure to do DNA fingerprinting on these microbes.

Tracking of microorganisms is an activity required in numerous critical situations on Earth. These include epidemiological investigations of local outbreaks, for instance in day-care centers, hospitals, and other closed environments such as office buildings. Such microbial tracking is not carried out often enough due to the requirements for specialized equipment and technical know-how that is not always present. The development and successful application of the REP-PCR methodology should simplify and expedite the tracking of microbes by DNA fingerprinting and make this type of investigation much more generally applicable. The Mir space station missions provide an excellent opportunity to develop and test methodology to follow microbial transfer in a situation that is of great interest to the space community, where the need to control infectious diseases is critical in a closed environment where there will not be access to extensive medical care. At the same time, this methodology, once proven, can be readily extended into the situations on Earth alluded to above.

FY97 Publications, Presentations, and Other Accomplishments:

Brauning, C.K.-F. An epidemiological evaluation of *Staphylococcus aureus* on two Mir missions using REP-PCR. (Thesis) University of Texas Houston Health Science Center (August, 1997).

Fecundity of Quail in Spacelab Microgravity

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Funding:

UPN/Project Identification: NAS2-14213

Solicitation: 93-OLMSA-06

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$14,400

Flight Information:

Experiment ID: 9306015

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Incubator II Joint US/Russian

Task Description:

The fundamental question of whether complete normal embryogenesis of the Japanese quail can be accomplished in microgravity was addressed with the recently completed NASA-2 STS-76-79 flight experiment on the Russian Space Station Mir. This study, a cooperative effort between Russian and American scientists, engineers, cosmonauts, and astronauts, was designed to study morphogenesis of several organs and systems in the Japanese Quail, *Coturnix japonica*. This PI concentrated on the differentiation and development of the reproductive system. The initial analysis of data suggests that the reproductive organs of flight subjects did not differ from the laboratory or synchronous controls. The gross morphological development appears to indicate that between 33% of the E-16 (fixation age in days) and 59% of the E-3 - E-16 of Flight embryos developed at a "normal" rate in space microgravity. Gross abnormalities were observed in 13% of the Flight embryos while none of the laboratory or synchronous controls showed abnormalities.

The hypothesis that a regenerative life support system can be provided in the "Spacelab Mir" for the Japanese quail is substantiated under the parameters of this experiment. Fifty-nine percent of the Flight Embryos reached the predetermined scheduled time of fixation. Approximately 33% (4/12) of the unincubated fertile eggs that were sent to Mir and subsequently incubated on Mir to 16 days of development showed normal male and female sexual development as well as the ability to initiate breathing. Although this would have to be tested, the ability to hatch is presumed from these data. A difficulty that may be encountered in the actual hatching process of these embryos is the finding that some Flight embryos had their head oriented in the small end of the egg. This may impede the hatching ability of the embryos. Gross observations of the gonadal tissues from those embryos that were fixed at the properly designated age indicated a normal reproductive development of both male and female embryos. Histological data is still being processed. A question not answered by this experiment is whether the initial development of the embryo (Stage I to Stage X -XII Eyal-Gilada and Kochev, 1970 and 1980) will proceed within the quail hen's oviduct as it does under the Earth's force of 1-G. Since these eggs were

fertilized and oviposited on Earth, the normal initial development to a 40,000 - 600,000 celled zygote with possible axis initiation had already occurred. Whether this normal process of development will occur in microgravity is not yet answered.

Our experiments are designed to foster reproduction in space microgravity. Additionally, we expect to gather substantial information on basic embryonic developmental processes. This biology will have a direct bearing on the understanding of embryogenesis and reproduction. Furthermore, we expect to gain a better interpretation about the role that Earth gravity and space microgravity have on cell and tissue migration during embryo development and differentiation. Embryonic cell migration is a primary reproductive interest. In all vertebrates, the germinal cells (future sperm and eggs) must migrate from outside the embryo to the gonads where they will proliferate and differentiate to form spermatogonia and oogonia. Some information may be forthcoming on the need for controlled turning during avian embryonic development.

Temporal-spatial embryonic development at critical embryonic ages, Stages I to X-XII, may or may not proceed normally in space microgravity. The fact that a greater percentage of the Flight embryos were teratomers would indicate that 1-G of Earth and turning of eggs during incubation might positively influence embryonic development.

Additional knowledge about embryogenesis, fertilization, and endocrinology in space will have long-term benefits to humans. The new technologies that may have benefits to other researchers are the gelatin ration (which contains both solid nutrients and total water) as well as extended holding periods for fertile eggs in a liquid environment to prevent dehydration before incubation.

Renal Stone Risk During Long Duration Space Flight

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Funding:

UPN/Project Identification: E651	Solicitation: 94-OLMSA-01
Initial Funding Date: 1995	Expiration: 1999
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$ 176,000	

Flight Information:

Experiment ID: 9401651
Flight Assignment: NASA-Mir-1B
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: UCD, BCR, In-flight Urine Storage Tube Kit, Dried Urine Chemistry Hardware

Task Description:

Human exposure to microgravity results in a number of physiologic changes. Among these changes are the alterations in renal function, fluid redistribution, bone loss, and muscle atrophy, all of which contribute to an altered urinary chemical environment. In-flight changes previously observed include decreased urine volume or increases in urinary calcium, phosphate, potassium, and sodium excretion which could all potentially exaggerate the risk of renal stone formation. The formation of a renal stone in-flight could have severe consequences for both the health of the crewmember and the mission success. This study involves pre-, in- and post-flight 24-hr urine collections to assess the renal stone-forming potential that exists during space flight and to determine how long after flight increased risk exists. Additionally, the impact of dietary factors on the urinary chemical composition will be assessed. From these data and from the renal stone data obtained from Space Shuttle flights, potential countermeasures will be selected and tested to minimize/eliminate the risk of renal stone formation during space flight.

This investigation was manifested for Mir-21, NASA-2, Mir-22, and NASA-3 and is currently continuing with the NASA-6, NASA-7 and Mir-25 missions. Preflight, in-flight and postflight urine and dietary analyses have been completed for the Mir-21, NASA-2, Mir-22, NASA-3 missions. The results obtained from these missions will be combined with the continuing studies and potential countermeasures will be assessed to reduce the risk of renal stone formation. Initial evaluation of the data suggest that hydration may be an effective countermeasure to reduce the risk of renal stone formation during and immediately after space flight. Dietary selection by each crewmember may also play a role in influencing the urinary chemical composition and the impact of the in-flight diets will be considered.

Lessons learned from these early investigations have led us in the last year to modify the current flight hardware to maximize the crewmember's time and the quality of the science returned from space flights. Currently in progress are analyses of the renal stone dietary and urinary data from the long duration missions, planning for the in-flight testing of pharmacologic countermeasures to minimize the risk of renal stone development, investigations into the effects of space flight on the naturally occurring urinary protein inhibitors to renal stone formation, and the first in-flight testing of the dried urine chemistry hardware investigation.

Approximately 12 percent of the Earth-bound population will develop a renal stone sometime during their lives. Assessing the renal stone risk during space flight may lead to a better understanding of renal physiology, dietary interaction with potential risk, and bone and mineral homeostasis. Initially, lessons learned from studies on Earth will be used to minimize the potential for renal stone formation in crewmembers exposed to microgravity. The first phase of this investigation will assess the direct effects of microgravity on this potential during long-duration space flight. Following this assessment, proven Earth-based therapies will be recommended to protect the health and well-being of the crewmembers.

Studying renal stone risk during space flight requires the development of new technologies and methods. Developing means to maintain sample integrity and minimize deterioration during sample collection and transport during space flight will also aid in the study of the Earth-bound population especially in rural and Third World populations. An advanced technology has been developed where the urine is dried on a filter card, uses no preservatives, and can be stored at ambient temperatures for extended periods of time. This advanced technology is scheduled as a technology demonstration on the NASA-6/NASA-7 and Mir-25 missions.

FY97 Publications, Presentations, and Other Accomplishments:

Chen, Y.M., Pietrzyk, R.A., and Whitson, P.A. "Quantification of urinary uric acid in the presence of thymol and thimerosal by high-performance liquid chromatography." *J. Chromatography*, vol. 763, 187-192 (1997).

Lane, H.W., Whitson, P.A., Putcha, L., Baker, E., Smith, S.M., Gretebeck, R.J., Schoeller, D.A., Davis-Street, J., Pak, C.Y.C., Pietrzyk, R.A., Nimmagudda, R.R., DeKerlegand, D.E., Stewart, K., and Bourne, D.W.A. "Extended duration orbiter medical project final report. *Regulatory Physiology*." NASA Publication (In Press).

Whitson, P.A. "Renal stone risk assessment in astronauts." Phase I Research Results Symposium, Gilruth Center, NASA Johnson Space Center, Houston, TX (August 5 - 7, 1997).

Whitson, P.A., Pak, C.Y.C., and Pietrzyk, R.A. "Renal stone risk during space flight." 12th Man in Space Symposium. *The Future of Humans in Space* (June 8 - 13, 1997).

Whitson, P.A., Pietrzyk, R.A., and Pak, C.Y.C. "Renal stone risk assessment during space shuttle flights." *J. Urology*, 2305-2310 (1997).

Neuro-Thyroid Interaction on Skeletal Isomyosin Expression in 0-G

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Funding:

UPN/Project Identification: 106-30-04

Solicitation: 93-OLMSA-01

Initial Funding Date: 1994

Expiration: not available

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$63,255

Joint Agency Participation: NIH/National Institute of Neurological Disorders and Stroke

Flight Information:

Experiment ID: 9301103

Flight Assignment: Neurolab (STS-90, April 1998)

Responsible NASA Center: Ames Research Center

Task Description:

The goal of this project is to examine the interactive role of gravity, innervation, and thyroid hormone (T₃) in the developmental programming of myosin heavy chain (MHC) isoform expression in neonatal rodent antigravity and locomotor skeletal muscle. The central hypothesis to be tested is that gravity exerts a profound influence on the development and maintenance of slow (type I) MHC expression in antigravity and locomotor muscle, such that in its absence, a significant number of muscle cells up-regulate the expression of fast MHCs due to an increased responsiveness to thyroid hormone. In contrast, the normal expression of the fast IIX and IIB MHCs are developmentally regulated independently of gravity, but require both the presence of an intact nerve and T₃ in order for these isoforms to reach full maturation in expression by replacing neonatal/embryonic MHC isoforms that are normally only expressed during fetal and early neonatal development. An additional objective is to determine whether muscle development, in the absence of gravity, creates a deleterious response whereby recovery from exposure to microgravity in the neonatal stage results in an irreversible effect on muscle mass and the pattern of adult myosin isoform expression. To test these hypotheses, both ground-control and space-flight rodents were allocated into the following subgroups: normal-control; denervated (DEN); thyroid deficient (TD); and DEN plus TD. The microgravity-exposed neonatal animals (along with the Nursing Dams) will be subjected to space flight aboard the shuttle (Neurolab mission). At recovery (and 3-4 weeks following recovery), flight animals and ground controls will be processed so that key muscles will be obtained to study MHC isoform expression at both the mRNA and protein level of analysis using electrophoretic, immunohistochemical, and *in situ* hybridization technology.

For FY 97, our activities centered on three fronts: 1) Refining techniques to simultaneously measure myosin heavy chain isoform mRNAs in small rodent muscles such as typically seen in neonatal rats. This technique has evolved to the point that we have submitted a paper to the *Journal of Applied Physiology*, which is currently in press and scheduled for publication in the October, 1997 issue. 2) Conducted an extensive ground-based study on the effects of thyroid deficiency and intact innervation on MHC expression in neonatal rats. This work defines the time course of events resulting in the adult MHC phenotype in rodent muscle and will serve, in part, as a reference to the Neurolab flight experiment. This paper has been completed and submitted to the *Journal of Applied Physiology*. 3) Participated in Experimental Verification Tests (November, 1996) and the Facilities Trial Run (August, 1997) to demonstrate that our technology and experimental design are appropriate for configuration and integration into the Neurolab Mission, now scheduled for April, 1998. A major milestone in these latter activities is our demonstration that we can successfully implant an osmotic pump system into the abdominal cavity of the nursing Dam to administer a drug (propylthiouracil) to make both the Dam and the neonate cohort thyroid deficient in order to examine this manipulation on the muscle system of both ground-based and flight-based experimental rats as outlined in the goals of our research project. Thus, we have advanced our experiment on all levels to demonstrate its readiness for flight operation.

In this flight project, we will be addressing fundamental issues concerning the role of gravity and in particular the interaction of gravity forces and thyroid hormone in the regulation of the pattern of skeletal MHC expression in rodent antigravity and locomotor muscle. Previous work on both ground-control and space-flight animals suggests that gravity plays a pivotal role in dictating the muscle's contractile protein phenotype. We feel that this dependency on gravity to control the properties of muscle will be even more dramatic when examined in the context of muscle development. The adult phenotype for contractile and hence functional capability evolves during post-natal development. We believe that gravity may be essential for establishing the expression of slow MHC in muscle fibers, which is essential for antigravity function. That is, in the absence of gravity during neonatal development, the slow MHC gene will not be turned on sufficiently to establish this property. Also, since thyroid hormone appears to be essential for the normal development of muscle mass and contractile phenotype, we want to manipulate thyroid state as well in ascertaining the interaction of thyroid hormone (or its absence) and that of gravity on the muscle maturation process.

These experiments will for the first time delineate how gravity impacts an important developmental and maturation process which affects muscle mass and locomotor performance. While this work will not address a specific disease per se, we feel that the environment of weightlessness creates a disease-like process such as muscle wasting (atrophy). The research in this proposal will address this topic indirectly by examining the potential retardation of muscle growth, differentiation, and gene expression in young animals. Finally, by studying the process of recovery, we will begin to delineate if there are critical periods in musculoskeletal development that are highly sensitive to the influence of gravity; thus, in its absence, the ability of the organism to reach normal maturity becomes impaired with potentially deleterious consequences from a motor performance perspective.

FY97 Publications, Presentations, and Other Accomplishments:

Wright, C., Haddad, F., and Baldwin, K.M. "Analysis of myosin heavy chain mRNA expression by RT-PCR." *J. Appl. Physiol.* (In Press).

Integration of Neural Cardiovascular Control in Space

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Funding:

UPN/Project Identification: 106-30 (E294)
Initial Funding Date: 1995
Students Funded Under Research: 0
FY 1997 Funding: \$118,000
Joint Agency Participation: NIH/NHLBI

Solicitation: 93-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9301294
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: LBNP, handgrip valsalva and cold press. dev., ECG transcranial doppler, etc.

Task Description:

The broad objective of this experiment is to explore and define the mechanisms by which the autonomic nervous system regulates the circulation to support tissue perfusion, particularly in the brain, during adaptation to microgravity and readaptation to 1-G. The primary hypothesis is that adaptation to the unique environment of microgravity minimizes the dynamic demands on the cardiovascular neural control. The level of physical activity is decreased, and no postural adjustments are required. This regulatory environment is likely to degrade important control mechanisms.

The experimental design represents an integrated approach to the testing of this primary hypothesis. The following questions will be answered: 1) Does efferent sympathetic nerve activity increase appropriately in response to baroreflex and non-baroreflex-mediated stimuli during and after space flight? 2) Can integrated clinical tests of autonomic function detect functional impairment, and can they be used to characterize the time course of adaptation to microgravity? 3) Does regulation of the cerebral circulation change in parallel with or independent of the regulation of the systemic circulation? 4) Can advanced mathematical models of neural control including both linear and non-linear dynamics be developed to gain insight into the integration among neurocirculatory variables and control mechanisms? A series of well-defined physiological stimuli has been defined, including lower body negative pressure, a cold pressor test, isometric exercise, Valsalva, and controlled

breathing. Responses are characterized by multiple measurements including heart rate, continuous finger arterial pressure and direct recording of muscle sympathetic nerve traffic.

Instrumentation has been defined, tested, and integrated. Detailed plans for supporting ground-based studies and for crew training have been defined and implemented. Crew training has included microneurography. Protocol validation included successful completion of the full cardiovascular R+0 protocol in 12 normal volunteers. A modified version was done in 10 additional subjects.

The experiment will provide new data on human cardiovascular control mechanisms. Orthostatic hypotension is a common and important condition in astronauts early after return from space and is also a common clinical problem. The experiment is likely to provide new and specific information on pathophysiological mechanisms, highly relevant to both general clinical practice and to flight medicine.

II. Program Tasks — Flight Research

Space Flight, Stress, and Neuronal Plasticity

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Funding:

UPN/Project Identification: 106-50-10

Initial Funding Date: 1994

Students Funded Under Research: 0

FY 1997 Funding: \$83,802

Joint Agency Participation: NIH/National Institute on Aging

Solicitation: 93-OLMSA-01

Expiration: not available

Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9301153

Flight Assignment: Small Payload (TBD)

Responsible NASA Center: Ames Research Center

Task Description:

When humans are exposed to the conditions of space flight for extended periods, a number of neuralgic disorders emerge. These pathological changes affect a wide variety of neuronal systems ranging from motor to hypothalamic to sensory function, and the effects can be long lasting. Such changes appear likely to involve both functional and morphological alterations in the brain, but the underlying mechanisms have been unclear. Recent work suggest that environmental influences including stress and altered hormone levels may influence neuronal morphologies and neuronal dynamics. The experiments in this application are intended to characterize the effects of space flight and elevated corticosteroids on the dynamics, organization, and composition of the neuronal cytoskeleton. Particular emphasis will be placed on the axonal transport, composition, and organization of the axonal cytoskeleton. The ability of pharmacological agents to block these morphological and functional changes will be determined. These studies will characterize the structural consequences of exposure to space flight and altered hormonal levels. A parallel set of studies will analyze functional consequences of these treatments by evaluating molecular mechanisms of vesicle trafficking in the presynaptic terminal important for neuronal plasticity and synaptic transmission. The goal of these studies is to determine the extent to which vesicle trafficking in the synapse contributes to functional plasticity. Pathways and molecular mechanisms involved will be identified, and changes associated with space flight and elevated corticosteroids will be characterized. The long-term goal of this research program is to provide molecular correlates for changes in functional architecture of the nervous system associated with long-term exposure conditions of space flight.

The primary flight component involves a study of stress on functional neuronal architecture in mice. component was a new initiative, substantial groundwork is needed before these studies are fully under present, we have established the ground-based studies and refined protocols for eventual application to flight animals. Baseline studies are now being done on control animals and steroid-treated animals. procedure for the administration of corticosteroids and drugs has been developed and will be refined

coming months. We have established the validity of ELISAs for quantitative analyses of cytoskeletal proteins in different brain regions. Significant differences have been found in the levels of several cytoskeletal proteins in steroid-treated animals. These studies are being extended to additional markers and regions of the brain. The Golgi impregnation method for visualizing neuronal and glial morphologies is being optimized and initial data collection on cell shape has begun. Initial results suggest a regional difference in morphological changes and raise the possibility of a biphasic response. Immunocytochemical studies and quantitative *in situ* localization of mRNA in brain sections are being initiated. Previous work on presynaptic function in the squid giant synapse is still underway.

The studies supported by this grant are intended to look at the effects of physiological stress on neuronal function and neuronal architecture. Previous studies have shown a number of deleterious effects on neuronal functional architecture associated with chronic stress. The conditions of space flight can result in stress of unusual duration, but physiological stress is commonly associated with a wide range of human activities. Many stress-related medical conditions have been documented. Since many of these changes appear similar to changes associated with the aging nervous system, these studies may also illuminate the mechanisms that lead to decrements in neuronal function with aging. The goals of these studies are: 1) to understand the molecular basis of neurological changes associated with stress, and 2) to devise treatments that can minimize deleterious changes in neurological function associated with chronic physiological stress.

Microgravity Effects on Developing Vestibular Afferents

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10	Solicitation: 93-OLMSA-01
Initial Funding Date: 1995	Expiration: not available
Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: \$84,787	
Joint Agency Participation: National Science Foundation	

Flight Information:

Experiment ID: 9301106
Flight Assignment: ARF-2 and Small Payload (STS-95, October 1998 [Target])
Responsible NASA Center: Ames Research Center
Flight Hardware Required: Aquatic Research Facility

Task Description:

This project will examine effects of the gravitational environment on the development of specific neuronal connections between vestibular sensory organs and their central nervous system targets in the zebrafish, *Brachydanio rerio*.

The proposed flight experiments, involving examination of primary vestibular afferents of zebrafish embryos raised in microgravity, will help determine the effects of altered patterns of neuronal activity on the development of connections in the vestibular system. In addition, these experiments may reveal an anatomical substrate for the observed plasticity in swimming behavior of fish seen during space flight.

The development of organotopic specificity in the primary vestibular afferent projection to the vestibular nuclei will be studied in zebrafish raised in three different environments: microgravity, 1-G centrifugation during flight, and 1-G ground-based control conditions. Lipophilic-dye fiber-tracing techniques will be used to label populations or single axons in specimens fixed at different ages. The pattern and extent of axonal growth of afferents from each vestibular sensory organ will be examined both in whole mount using confocal microscopy and in cryostat sections. These experiments will be the first to study the effects of microgravity on the development of the neuronal connections underlying vestibular senses, as well as to document the normal development of these connections. Flight experiments will provide data on the relative role of patterns of neuronal activity versus inherent positional cues and tropic factors in the development of specific connections in the vestibular system.

During the past year we have continued our efforts to adapt our scientific protocol to the flight hardware (the Canadian Space Agency's Aquatic Research Facility (ARF)). Progress has been made in two areas: first, we

have completed survivability tests and determined that we can keep 40 zebrafish embryos alive and developing normally for flight duration tests in the small aquaria available in the ARF. We will be able to use 6 aquaria in both the 1-G and 0-G flight experiment groups (embryos in each separate aquarium will be fixed at different times during the flight). The 40 embryos/aquarium should ensure that we will be able to collect enough data for statistical significance at each fixation time point. Second, we have developed a fixation protocol which is compatible with both our experimental objectives (labeling the primary vestibular afferents of zebrafish from the flight experiments with lipophilic dyes to determine effects of microgravity on their development) and with the ARF which provides automatic fixations during flight, but which (for safety reasons) does not allow the use of any flammable fixative. We have found that in-flight fixation with Histochoice (Amaresco) followed by post-flight post fixation in 4% paraformaldehyde provides good preservation of the tissue and allows for the transport of lipophilic dyes. Unfortunately the Histochoice does make the tissue more opaque than do other fixatives, so we are currently working on clearing protocol for further processing the specimens post-flight in order to better visualize the labeled axons.

Additionally during the past year we have continued our ground-based studies of the normal development of vestibular afferents and the extensive re-modeling of these afferents which occurs during the first few days of life in the zebrafish embryo.

Normal development of the vestibular system at 1-G results in an adult projection pattern of the vestibular nerve onto the vestibular nuclei, which is similar in all species of vertebrate studied. In all cases, primary vestibular afferents serving the different vestibular end-organs have distinct though overlapping patterns of axonal arborization in the vestibular nuclei. Although this adult pattern of organotopically organized projections has obvious advantages for sensory processing, little is known about its normal development. Knowledge of the mechanisms responsible for the development of the normal pattern of connectivity can be gained by studying use- or environment-dependent changes in vestibular system development.

The adult pattern of vestibular afferent projection, with inputs from each of the semi-circular canals and otolithic organs occupying specific regions of the vestibular nuclei, could arise from a variety of different development mechanisms. Developing vestibular axons serving the different end organs could be guided directly to their targets by molecular positional cues or trophic factors, or the axons could initially form overlapping terminal arbors and later segregate based on a competitive neuronal-activity dependent process. Previous work from many laboratories studying the development of the visual system in a broad range of vertebrate species highlights the importance of patterned neuronal activity in the establishment of specific neuronal connections in that sensory system. Space flight offers the opportunity to study the development of connections in the vestibular system under conditions where the normal patterns of neuronal activity in the system are disrupted by the absence of the normal influence of Earth's gravitational field.

Examination of the patterning of the primary vestibular afferent projections in animals raised under conditions of microgravity will disclose the role that neuronal activity plays in the development of the vestibular system and will thus help determine whether the lessons learned from studies of the visual system can be generalized to developmental rules for other sensory systems. In addition, these studies of the experience-dependent changes in axonal arbors in the vestibular system may reveal the anatomical substrate of the behavioral adaptation to microgravity which occurs after a few days in space. Because the vestibular system exhibits an extreme degree of evolutionary conservation, much of what is learned from the proposed experiments about the vestibular system of the fish should be applicable to the vestibular system of higher vertebrates, including humans. Whether we find that activity-dependent changes in the anatomy of primary vestibular afferents in zebrafish raised in microgravity do occur, or whether no such changes are seen, our experiments should help to answer basic questions about the role of neuronal activity in the development of specific connections in sensory systems.

Adaptation to Linear Acceleration in Space (Atlas) - Spatial Orientation of Vestibulo-Ocular Reflex and of Velocity Storage

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Funding:

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Students Funded Under Research: 0
FY 1997 Funding: \$697,085

Solicitation: 93-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9301047
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Johnson Space Center

Task Description:

The yaw axis component of optokinetic nystagmus (OKN), optokinetic after-nystagmus (OKAN), and the vestibulo-ocular reflex (VOR) tends to align with gravity on Earth in monkeys and humans. After space flight, the yaw component of the VOR of monkeys moves toward a body rather than a gravitational frame of reference. From this it is postulated that adaptation to space causes a shift in the orientation vectors of OKN and the VOR from a gravitational to a body reference frame. How orientation is altered by introduction of linear forces in space is not known. Critical experiments are proposed in Neurolab to determine how microgravity affects the orientation vectors of the VOR and of OKN, and how they are altered by introduction of linear forces due to centrifugal acceleration in an eccentric rotator. Linear accelerations of 1-G and 0.5-G will be introduced along the subject's head interaural axis (left-ear-out or right-ear-out) and along the subject's yaw axis, inducing roll. OKN will be induced by a binocular optokinetic stimulator with subjects stationary and during centrifugation to determine whether the yaw axis component of OKN aligns with gravito-inertial acceleration (GIA), as on Earth, or with the body axis. The orientation of pursuit contributions to the OKN response will be evaluated by combining eccentric rotation with a smooth pursuit stimulus produced by movement of a small target on the screen of the binocular optokinetic stimulator. Eye movements will be recorded by a binocular three-dimensional video technique. Subjects will report their subjective motion and/or orientation sensations

during centrifugation in darkness. The axes of eye rotation will be calculated using a model-based approach. Tilt in a tilt-chair will be used in ground-based testing to induce ocular counter-rolling (OCR). The astronauts will also be tested preflight and postflight during static head tilt relative to gravity. These experiments will help in understanding how spatial orientation and OCR are altered in microgravity and in designing tasks and countermeasures in space and on re-entry.

During FY 1997 the following has been accomplished:

- 1) Flight and Training Equipment: The training centrifuge Body Rotating Device (BRD), which was built by Aerospatiale under ESA jurisdiction was installed in the mockup at JSC and is now being used for training. The flight centrifuge BRD was sent to KSC to prepare for flight. The ground-based rotator, which will be used for our ground-based testing and for the pre and postflight testing, will be delivered to Mount Sinai School of Medicine in the first week of November of 1997.
- 2) A new piece of equipment was added to our list: a tilt chair with optokinetic stimulator. This will permit us to test OCR and the response to optokinetic nystagmus as a function of gravity on Recovery Day 0. The tilt chair was extensively tested at Mount Sinai School of Medicine and then disassembled. It will be shipped, via Neurokinetics, Inc., to the Kennedy Space Center. Perceptual tests on the ability to determine the spatial horizontal and vertical will also be performed on recovery day on this apparatus.
- 3) Eye movement recording has been refined. The EMRS that will go on the rotator that we are building to come to Mt. Sinai is still in France and is being used to help get the TPF to a functional state. The tape processing facility (TPF) is not ready yet. Continuing effort has gone into improving the eye images recorded by the EMRS and into the TPF that will be used to measure eye movements from the video images. Dr. Steven Moore, from our laboratory, has been traveling between New York, Grenoble, and Houston to help LETI, the French company, accomplish the TPF.
- 4) The software that will be used in the flight has been finished and tested.
- 5) The procedures have been finalized for the Crew.
- 6) We accomplished the science verification test of the flight equipment at JSC.
- 7) We participated in Crew Training throughout the year.
- 8) We participated in a Mission Integrated Training Session (MITS) of Flight Day 7.
- 9) We tested Jean Loup Cretien, a French astronaut, who is currently flying, and he will be tested upon his return.

In a preliminary analysis, we were able to demonstrate that the axis of eye rotation shifts during yaw rotation on a centrifuge. This demonstrated that the apparatus can induce shifts of the axis of eye rotation that can be tested in flight.

This research will determine how otolith-ocular reflexes and spatial orientation of the angular vestibulo-ocular reflex are altered after adaptation to space. This information will be used to understand deficits in gaze and posture that occur when astronauts adapt to microgravity and then readapt to the 1-G terrestrial environment of Earth. The information will also be used to direct countermeasures to overcome lags in adaptation or changes in gaze and balance due to the abnormal force field environment of microgravity. Such information and countermeasures will be critical for long-duration space flights to the Moon or Mars.

We previously found that there was prolonged depression of OCR after adaptation to microgravity. If this depression of torsional eye movements is present in space, it is important to recognize it and to limit tasks that

might require such eye movements. If it is present in humans after space flight, it will be necessary to consider countermeasures by which normal OCR can be restored after landing to minimize postural and gaze deficits.

Vergence is essential for good fixation when moving toward visual targets. We found in the COSMOS project that there was prolonged depression of vergence in response to naso-occipital linear acceleration in the monkey. These findings are provocative but incomplete in that only two subjects were recorded. We will collect data on vergence in the present experiments. If there were problems with verging the eyes while moving toward targets after landing, it could have important functional significance.

A major advance will be in the development of a three-dimensional model of the VOR which will include both angular and linear acceleration inputs and which will account for dynamic changes that alter the orientation of the system vectors to those of gravito-inertial acceleration. This will provide fundamental understanding of how processing of otolith information and spatial orientation are altered in the absence of gravity.

Findings from space research can readily be applied to human disorders on Earth. First, we will gain understanding of how spatial orientation is disrupted in conditions in which there is postural imbalance or gaze instability. A simple example where such information will have important clinical significance is in understanding postural imbalance of the elderly. Our hypothesis is that an important source of imbalance in patients with lesions of the brain or vestibular apparatus is a failure of alignment of orientation vectors with the summed vector of linear acceleration.

We are developing a new three-dimensional, binocular video technique for recording eye movements that has great potential clinical significance. It is readily applied, non-invasive, and highly accurate, and should become the method of choice for studying patients with vestibular and oculomotor disorders.

FY97 Publications, Presentations, and Other Accomplishments:

Dai, M., Raphan, T., Kozlovskaya, I., and Cohen, B. "Vestibular adaptation to space in monkeys." *Otolaryngol., Head & Neck Surg.* (In Press).

stages and spectral composition of the EEG during sleep will be determined as well as its effects on daytime alertness and performance.

During FY97, through continual collaboration with the Experiment Support Scientist (ESS), Payload Project Manager, and other Sleep Team members at UCSD, NASA, and Lockheed Martin, and in collaboration with the PI at NASA Ames and MIT, we have conducted several training sessions, and continued the refinement of hardware and software.

The training sessions have been successful and the crew is now proficient in sleep instrumentation, cognitive performance testing, and temperature collection. In addition, malfunction training has resulted in a crew that can handle many malfunctions related to the sleep recordings and cognitive performance testing. During these training sessions, the crew members have provided us with positive feedback and many suggestions concerning the hardware and software. In response to these suggestions, we have modified both hardware and software.

In particular, the sleep-nets underwent modifications to make them more comfortable. The firmware of the digital sleep recorder was modified so that patient ID numbers and Mission Elapsed Time can be entered easily.

The neurobehavioral assessment battery software was modified. In addition, new software was designed to replace the sleep logs on paper.

In continuous collaboration with the "PI in a box" team, we have improved the computerized display of polysomnographic data and the computerized signal quality detection.

At the outset of this project, it was planned to record body temperature from a rectal sensor. On previous space flights, the use of this method was unsatisfactory. The procedure has been modified to a method in which the subject swallows a pill which emits radio frequencies that are temperature sensitive. The crew identified a new recorder (called the BBN), which was developed at the Natick Army laboratories. In collaboration with Dr. Reed Hoytt, a new software package was designed and written which will allow the crew display the body temperature on screen. This new software and hardware will greatly facilitate the long-term recording of body temperature during space flight.

The investigative team attended two Investigator Working Group (IWG) meetings. During both IWGs, issues related to baseline data collection and inflight time lining were discussed.

At the end of FY 97, the software and hardware development was completed and we are prepared to begin baseline data collection.

This work holds promise for the development and identification of a novel, safe, and effective hypnotic. This would have widespread applications, particularly among groups with a high prevalence of insomnia, such as shift workers and the elderly. Use of the naturally occurring hormone melatonin as a hypnotic has many potential advantages as compared to currently employed pharmacologic agents. The extent of melatonin's effects on mood and performance are approximately the same as those produced by administration of clinically efficacious doses of hypnotic drugs such as the benzodiazepines. However, unlike the benzodiazepines, melatonin does not appear to impair memory either immediately after administration or the next day. In addition, residual effects of melatonin on vigilance, reaction time, and alertness do not appear to be present following its use as a hypnotic, although such effects are well documented following administration of many benzodiazepines. Therefore, regardless of melatonin's physiological functions, its use as a hypnotic may have advantages over currently available pharmacologic agents. Actually, at least five major pharmaceutical companies are developing plans for clinical trials of the hypnotic effects of melatonin for the treatment of insomnia.

Autonomic Neuroplasticity in Weightlessness

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Funding:

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Initial Funding Date: 1996 Expiration: 1999
Students Funded Under Research: 0 Post-Doctoral Associates: 3
FY 1997 Funding: \$210,791
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

Experiment ID: 9301049
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Johnson Space Center

Task Description:

Astronauts return to Earth with modestly reduced blood volumes, abnormal reductions of arterial pressure and increases of heart rate with standing, and substantial impairment of vagally mediated arterial baroreflexes. We propose studies in astronauts to test the hypotheses that 1) baroreflex malfunction after weightlessness is a consequence of neuroplasticity occurring during weightlessness and that 2) autonomic responses to acute and chronic blood volume reductions can be documented, and the mechanisms which cause such responses can be defined.

A unique aspect of this research is that muscle sympathetic nerve traffic will be measured directly in astronauts during blood volume shifts and actual head-up tilt. For the first time, muscle sympathetic nerve activity will be recorded in space. Baroreflex malfunction after weightlessness as well as autonomic responses to reduced blood volume will be investigated with controlled frequency breathing, arterial baroreceptor reflex responses, spontaneous arterial pressure and R-R interval fluctuations, lower body negative pressure, passive 60° head up tilt, ramped/graded neck pressure and suction, and valsalva maneuvers.

Subtle changes that occur at microgravity may physiologically become highly significant after return to the 1-G environment. There are compelling general scientific reasons to take advantage of the access to microgravity to study the dynamic aspects and integration of neural regulation of the cardiovascular system. The unique environment of space with the absence of hydrostatic gradients and the reduction in the overall level of physical activity drastically alters the operating conditions of the circulatory system. Analysis of the effects of

microgravity on specific aspects of neural regulatory mechanisms as proposed in the present study has the potential to produce new information on properties of physiological control mechanisms.

During the past year, considerable effort was devoted to refining the inflight experimental protocols. As part of this process, our Virginia-based group conducted several studies to compare various methods for implementing the controlled frequency breathing experiments. Subsequently, the four investigator groups that make up the Autonomic Team finalized specific details of the controlled frequency breathing experiments. In addition, other important details of the inflight protocols were also finalized. The Autonomic Team also devoted considerable effort to refining the software that will be used during the inflight experiments to prompt the astronauts through the inflight protocols. This software also controls several critical pieces of equipment that have to be activated at precise times, making it extremely important. We worked with the software engineers at Johnson Space Center who developed this software to optimize the software.

Two very important ground studies were conducted during the past year as a team effort by the Autonomic investigators. In January, we carried out a ground study, hosted by our Vanderbilt colleagues, in which volunteers were run through the late-mission portion of the Autonomic inflight protocol. In September, we participated in a study hosted by our Dallas colleagues to gain experience in carrying out our experiments on landing day. Both of these ground studies were highly successful. Ground-based simulations of selected flight days are also being carried out by the payload specialists at NASA's Spacelab Mockup. To date, two of these simulations have involved Autonomic Team experiments.

This research will address issues of great physiological and pathophysiological interest. First, it should improve understanding of a basic physiological mechanism: human cardiovascular autonomic responses to standing upright. Second, it should improve understanding of pathophysiological mechanisms of enormous public health significance. For example, hypertension, which afflicts over 60 million Americans, is associated with impairment of autonomic cardiovascular control. Another example is acute myocardial infarction and a closely related problem, sudden cardiac death. Sudden cardiac death is the largest cause of death in developed countries; the number of people who die suddenly of catastrophic dysrhythmias dwarfs the number of people who die of other public health problems, including AIDS, which attracts much more media attention and research funding. In cardiac patients, abnormal autonomic cardiovascular control (as reflected by impairment of baroreceptor-cardiac reflexes and reduced heart rate variability) indicates which patients are at greatest risk for subsequent cardiac events. Therefore, understanding of how autonomic cardiovascular control mechanisms become impaired may be very important. It is the nature of human research that patients with pathologic conditions are not evaluated *before* they become ill. (Physicians who would study such patients do not know who will become ill.) Therefore, astronauts present a great opportunity: they can be studied before space missions when they are normal; in space, as they become abnormal; and after return to Earth as they become normal again. Such longitudinal evaluation of patients is not possible.

FY97 Publications, Presentations, and Other Accomplishments:

Airaksinen, K.E.J., Tahvanainen, K.U.O., Kuusela, T.A., Huikuri, H.V., Niemela, M.J., Karjalainen, P., and Eckberg, D.L. "Cross spectral analysis in assessment of baroreflex gain in patients with coronary artery disease." *Ann. Noninvas. Electrocardiol.*, vol. 2, 229-235 (1997).

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Seidel, H., Herzel, H., and Eckberg, D.L. "Phase dependencies of the human baroreflex." *Am. J. Physiol.*, vol. 72, H2040-H2053 (1997).

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Vesalainen, R.K., Kaila, T.J., Kantola, I.M., Tahvanainen, K.U.O., Kuusela, T.A., Eckberg, D.L. "Low-dose transdermal scopolamine in mild essential hypertension." *J. Hypertens.* (In Press).

Vesalainen, R.K., Tahvanainen, K.U.O., Kalia, T.J., Kantola, I.M., Kuusela, T.A., and Eckberg, D.L. "Effects of low-dose transdermal scopolamine on autonomic cardiovascular control in healthy young subjects." *Clin. Physiol.*, vol. 17, 135-148 (1997).

CNS Control of Rhythms and Homeostasis During Spaceflight

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Funding:

UPN/Project Identification: 106-30-04	Solicitation: 93-OLMSA-01
Initial Funding Date: 1994	Expiration: not available
Students Funded Under Research: 6	Post-Doctoral Associates: 0
FY 1997 Funding: \$93,490	
Joint Agency Participation: NIH/National Heart Lung and Blood Institute	

Flight Information:

Experiment ID: 9301132
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center

Task Description:

Animals have evolved and developed within the constant gravitational environment of the Earth and the dynamic changes in the environment associated with the 24-hour day. A key element in the evolution of mammals was the development of homeostasis, the ability to maintain a relatively constant internal environment. An evolutionarily older adaptation was the development of the ability of organisms to temporally coordinate their physiology and behavior both internally and with the external day. The circadian timing system (CTS) is an important temporal organizer controlling both physiology and behavior. The importance of proper CTS function is illustrated by the fact that conditions such as jet lag, shift work, and some sleep and mental disorders are frequently associated with dysfunction of the CTS. Animals exposed to the microgravity environment of space flight exhibit alterations in both CTS function and homeostasis. These alterations have included changes in body temperature regulation and metabolism, changes in the timing of physiological and behavioral functions, fragmentation of the sleep-wake cycle and even desynchronization of some rhythmic variables from the external light-dark cycle. In addition, our previous studies have shown that exposure of both mature and developing animals to hyperdynamic fields via centrifugation significantly affects both the CTS and homeostasis. This research will examine the physiology of the CTS and homeostatic control systems of animals exposed to space flight. These studies will examine the effects of space flight on four areas: (1) circadian rhythms; (2) neural responses of the circadian pacemaker and the sensory pathway for light information from the retina to the CTS; (3) adaptations in homeostatic regulation; and (4) neural changes in hypothalamic nuclei that regulate specific homeostatic functions. We will thus be examining the effects of space flight on selected physiological systems and on the central neural controllers of the same systems.

During FY97, several specific aims of our research have been accomplished. Our studies have continued to examine the circadian rhythms of Fischer 344 rats in LD cycles and constant red light. We have also supported several project activities, including a RAHF Housing Test at Ames Research Center and the Facility Trial Run

(FTR) at Kennedy Space Center. FTR validated all of our major procedures to be performed at Kennedy to support our experiment for the flight.

We demonstrated that a one-hour phase-shifting light pulse will induce significant c-Fos expression within SCN neurons in Fischer 344 rats. We further tested the light pulse protocol to determine the effects of duration, intensity, and timing of a light pulse for initiating c-Fos expression in the SCN of rats in order to determine the optimal flight protocol.

We have continued to test the flight hardware so that it will be adequate for rhythm analysis. These tests have included long-term recordings from rats implanted with biotelemetry transmitters like those that will be used in the flight experiment. Our tests have allowed us to refine the surgical procedure used to implant the transmitters as well as the transmitter design. Further analyses of the data have allowed the engineers to improve the quality of the data collection system.

We have also continued to refine our immunohistochemistry protocols in order to improve the staining.

Space flight has taken humans and animals into a new environment, removed from Earth's normal gravitational field and daily cyclic fluctuations. These environmental changes induce an adaptive response in many physiological systems that may temporarily or permanently result in dysfunction. For example, Apollo astronauts experienced perceptions of cold discomfort, even though body and ambient temperatures remained in the normal range. Whether the perception of cold discomfort was due to gravitational effects on thermoregulatory mechanisms or possible desynchrony of temperature rhythmicity induced by abnormal circadian rhythms is not known. Another example is that of space adaptation syndrome which is primarily thought to involve microgravity's effect on vestibular and kinesthetic sensory systems. Further, desynchronization of circadian rhythms during space flight may contribute to this adaptation and result in physiological discomfort analogous to jet lag. Surveys reveal that most crew members suffered from sleep disruption during the missions, while cosmonauts on long-term missions appear to have been particularly vulnerable to the effects of fatigue. It is thus not surprising that some astronauts use sleeping pills. Misalignment of circadian rhythms may play a prominent role in these disturbances. These few examples demonstrate that the biomedical problems of space will require an examination of the respective contribution of gravity and circadian rhythmicity to these adaptation syndromes.

Chronic Recording of Otolith Nerves in Microgravity

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Funding:

UPN/Project Identification: 106-30-04

Solicitation: 93-OLMSA-01

Initial Funding Date: 1994

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Students Funded Under Research: 1

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FY 1997 Funding: \$86,391

Joint Agency Participation: National Science Foundation

Flight Information:

Experiment ID: 9301088

Flight Assignment: Neurolab (STS-90, April 1998 and STS-95, October 1998)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: almost complete, being constructed by Japanese colleagues, Mitsubishi

Task Description:

The overall goals of the proposed research are to study the effects of microgravity on the response dynamics of the afferents of the utricle and saccule and to study any activation and action of the efferent vestibular system related to the microgravity environment. We will utilize toadfish, *Opsanus tau*, with multichannel wafer electrodes placed in the nerves innervating the saccule and utricle. These electrodes will be chronically implanted into a small cut made in these nerves, and individual axons will regenerate through the pores in the electrode to yield chronic recordings. We will record the responses of both primary afferents and central nervous system efferent fibers. We will characterize responses in normal gravity and in microgravity. Because we will record from the same fibers in both environments, we will have a measure of the effects of reduced gravity upon the performance of the otolithic organs and will assess whether the microgravity environment leads to the activation of the efferent vestibular system. Results of these experiments will bear upon theories that invoke the action of the efferent system as one of the etiologies of space adaptation syndrome. Further, studies of the cellular and systems science aspects of the vestibular system and its efferent control add information about function and may bear upon future therapies and mechanisms for the control of Earth-bound motion sickness.

Progress in the use of the wafer electrode has been made. Wafers were coated electrostatically with an artificial "silk" fiber less than 1 micron in diameter. This fiber has laminen incorporated into its matrix. Laminen is a factor to promote the growth of neurites. Histological examination of the wafer electrodes with confocal microscopy in conjunction with neuron-specific antibody stains indicated the nerve fibers had regenerated and grown through each and every pore in the wafer electrode. We are presently testing other subjects implanted with this laminen coated wafer for neural activity.

Progress has also been made in the development and implementation of the electronics for this experiment. The fish-mounted transmitter now appears functional and tests are proceeding.

We have a long-term commitment to the study of the acousticolateralis system in the toadfish, *Opsanus tau* and have studied this system extensively. Fish vestibular systems compare favorably with those of other animals. Vestibular organs, particularly the semicircular canals, were highly evolved when vertebrates first appeared; their function has not appreciably changed. Bode plots that describe canal response dynamics are remarkably similar across the vertebrate phyla. Inter-species differences appear to be related to the lifestyle of the particular animal reflecting the range of angular and linear accelerative forces experienced. Thus, we expect that our results, obtained from fish, will bear directly on the human condition.

The saccular and utricular maculae of the vestibular system primarily sense the linear acceleration vector consisting of gravitational and inertial components. We propose to chronically record otolithic afferent responses in freely moving animals before, during, and after space flight to assess the effects of microgravity. Because this experiment will include the results of the gravitational unweighting of the otolithic mass, we should be able to delineate the effects of the inertial and gravitational components of the acceleration vector. Otolithic organ morphology and physiology has been highly conserved throughout evolution. Thus, these results should mimic the identical physiology occurring simultaneously within the ears of the astronauts accompanying our fish in the NASA shuttle. We hypothesize that there will be changes in the firing pattern of otolithic afferents when the otolithic mass is "unweighted" in microgravity; inertial responses should be unchanged.

There are profound interactions of the vestibular system with all of the body's sensory, motor, vegetative, and cognitive functions. These interactions begin with the vestibular end organ that senses the linear acceleration vector consisting of gravitational and inertial components. This information travels to the brain via the VIIIth cranial nerve to allow computations about dynamic and static position of the head. Knowledge about the variability in the function of the linear accelerometers resident in the inner ear in parallel with variations of the gravity vector will add information that has profound implications for vestibular and other bodily functions. Further, space adaptation syndrome presumably begins with "aberrant" information about the gravity vector originating within the inner ear. Those animals lacking a labyrinth do not manifest space adaptation syndrome or motion sickness. The central nervous system also contains neurons that are "efferent" or project from the brain to the labyrinth to modify incoming information before it reaches the brain. Previous extensive experiments upon the efferent vestibular system have led to the characterization of its effects upon the labyrinth. Because we will record from the same otolithic fibers in normal and in microgravity, we will have a measure of the effects of reduced gravity upon the performance of the otolithic organs and will also be able to assess whether the microgravity environment leads to the activation of the efferent vestibular system. Results of these experiments will bear upon theories that invoke the action of the efferent system as one of the etiologies of space adaptation syndrome. Results concerning space adaptation syndrome may also apply to terrestrial motion sickness.

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Funding:

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Joint Agency Participation: NIH/National Institute on Deafness and other Communication Disorders

Flight Information:

Experiment ID: 9301127

Flight Assignment: Neurolab (STS-90, April 1998)

Responsible NASA Center: Ames Research Center

Task Description:

Exposure to microgravity causes postural, locomotor, and oculomotor modifications. In order to realize long-term space flight, effective countermeasures for these abnormalities must be developed. Toward this end, it is essential to understand the cellular and biological basis underlying centrally mediated vestibular adaptation to altered gravity conditions.

The objective of the proposed research is to identify the morphologic alterations in rat cerebellar cortex that correlate with sensory and motor adaptation to microgravity. We propose ground-based and space-based studies to test the hypotheses that (a) ultrastructural alterations accompany adaptation to microgravity, and (b) such alterations are pathway- and neurotransmitter-specific. The merit of this idea has been emphasized in several brief communications by Krasnov and co-workers, in which ultrastructural changes in Purkinje cell synaptology have been reported in the nodulus of rats following space flight. These observations are of particular interest because Purkinje cells in the nodulus control habituation of the vestibulo-ocular reflex and are likely to be critical for maintaining spatial orientation with regard to gravity. In addition, physiologic investigations have clearly indicated a role for the flocculus in controlling specific aspects of the VOR.

We propose to study the cerebellar cortex from: (1) brain tissue already processed in our laboratory from flight and control rats of PARE.0.2 from the STS-54 shuttle mission; (2) flight and control rats from the Neurolab shuttle mission; and (3) naive laboratory rats. The tissue will be used for quantitative ultrastructural and immunocytochemical studies of synaptic circuits in the nodulus and ventral uvula, flocculus, and paraflocculus, and non-vestibular cerebellar cortex. We expect to obtain stereological data supporting a change in synaptology in vestibular, but not in nonvestibular, cerebella of flight rats. The qualitative and/or quantitative differences in excitatory amino acid and GABAergic neurotransmission in the nodulus and flocculus of flight rats will also be compared to controls and naive animals.

We expect to obtain critical information about the alterations in synaptology and neurotransmitter localization in the nodulus and flocculus that accompany adaptation to microgravity. The identification and characterization of GABAergic and GABA-receptive elements in this paradigm should lead to a greater understanding of how inhibition is modified in neuronal circuits during behavioral adaptation. Similarly, delineation of the microgravity-induced alterations in excitatory glutamatergic transmission will contribute to our basic knowledge of the morphologic basis for cerebellar-mediated motor learning. Through comparison of tissue from ground-based rats with animals sacrificed postflight and animals sacrificed during flight, it will be possible to localize, characterize, and quantify the site(s) and synapses that mediate vestibular adaptation phenomena in space.

Six methodological studies were conducted to evaluate strategies for quantitative analysis of cerebellar ultrastructure and immunocytochemical staining. Based on the results of these studies, we have established the fixation protocol, and the decision rules for tissue selection and electron microscopy that will be utilized for the experiments.

The six studies were completed in time to apply their conclusions to the Experiment Verification Test (EVT) held at NASA/Ames Research Center (ARC) in November, 1996. This test involved three experimental groups that were representative of the subject groups planned for the Neurolab shuttle. They included a Flight group, a Vivarium group, and a Hypergravity group. To simulate hypergravity at 2 times against the Earth's gravitational force, this latter group was placed in the 24-ft centrifuge facility at ARC. The 24-foot centrifuge consists of a central vertical shaft spindle driven by a 25 horsepower motor. Attached to the top of the spindle, approximately six feet from the ground, are ten radial arms. Each arm holds two enclosures 23.5" high x 39.5" wide x 22" deep. The centrifuge was set at 19.99 RPM to create a 2-G environment at the floor of the animal cages. As with the planned shuttle mission, each experimental group above had four sets of subjects: Flight Day (FD) 2 (N = 4), FD 14 (N = 9), recovery (R) day +1 (N = 4), and R+13 (N = 7). However, the centrifuge was stopped after 14 days, rather than the 17 days that were initially planned. The cerebellum from each of these 72 animals was obtained according to the tissue sharing agreement detailed in the Experiment Requirements Document. These samples were immersion-fixed according to the established protocol, and then Vibratome-sectioned. The medio-lateral extent of each cerebellum was measured before cutting to obtain an index of tissue shrinkage. All cerebella were sectioned in the sagittal plane, and the sections were collected serially, in order to correlate the medio-lateral position of each section with the parasagittal zones of the cerebellum established by other investigators. Once cut, each cerebellum was processed for electron microscopy and resin-embedded between plastic coverslips. The last of these samples was removed from fixative in late December 1996, and resin embedment of all samples was completed by mid-February 1997.

The analysis of this material was begun by examining tissue from the FD2 animals. The wafer-embedded cerebella from the three sets of animals (flight group, vivarium group, and hypergravity group) were photographed and then sketched for documentation. Then, a single sample of non-vestibular cerebellar cortex from each animal of these three groups was thin-sectioned to provide a sample of tissue condition. The three best-preserved cerebella from each group were selected for quantitative analysis.

The total number of sections from each cerebellum varied between 92 and 108, depending on the animal. All wafers were examined by light microscopy to identify the ones containing the nodulus. Initially, the two wafers (left and right vermis) containing the lateral-most sections through the nodulus were identified. An area containing cerebellar cortex from the ventral nodulus was then dissected, mounted on a blank resin block, trimmed, thin-sectioned, examined by electron microscopy, and photographed.

Each micrograph was coded, and evaluated by two investigators for the presence of Purkinje cell dendrites, climbing fiber terminals, parallel fiber terminals, and synapses between each of these bouton types and the Purkinje cells. The area of each of these neuronal elements, and the lengths of the synapses, are currently being measured for subsequent statistical analysis. To date, 12,000 μm^2 of tissue from the vivarium group, and 40,000 μm^2 of tissue from the hypergravity group have been analyzed.

Evaluation of this tissue will continue during the following year of the project. The experience gained during the conduct of the EVT experiment provided critical information for the laboratory logistics needed for the flight experiment. In addition, the scientific results of the hypergravity experiment are providing important guidance for focussed study of the tissue from the flight experiment.

This research will yield basic neuroanatomical and neurotransmitter information that will enhance current understanding of the vestibulo-cerebellum, and will clarify the role of defined cell groups and amino acid neurotransmitters in processing gravity-related information in the central nervous system. These studies will identify structural and neurochemical bases for the neuronal and synaptic plasticity that accompany CNS responses to altered gravitational environments. The studies are designed to provide insight into the morphologic and molecular changes that may occur in the brain during and following exposure to space flight, and to advance our understanding of the role of gravity in the maintenance of normal vestibular circuitry. Moreover, the results of these studies will contribute to our knowledge of the morphological basis for cerebellar-mediated adaptation and motor learning.

Effects of Space Flight on Drosophila Neural Development

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Joint Agency Participation: National Science Foundation

Flight Information:

Experiment ID: 9301042

Flight Assignment: NIH-B1 (STS-95, October 1998 [Target])

Responsible NASA Center: Ames Research Center

Task Description:

This project will examine the development of synaptic connectivity under the conditions of microgravity and space flight. The analysis will be confined to two motoneurons, the cells RP1 and RP3, and their three targets, muscle fibers 13, 7, and 6. More is known about the development of these two cells than for any other neurons in *Drosophila*. As a result, the RP neurons will serve as excellent benchmarks to determine whether there is any effect of microgravity on the development of individual neurons and identified synapses. Even subtle defects and targeting errors will be readily detected. In the seven abdominal segments from A1 to A7, there are paired sets of RP neurons, with each set innervating targets in the contralateral half-segment. All the sets of RP neurons behave identically. The motoneurons follow the same trajectories and choose the same segmentally homologous synaptic targets. Thus we will be able to examine synaptic development with single cell resolution in a large sample set of neurons. This will improve the accuracy of the planned morphometric characterizations. Finally, as the development of these neurons is very rapid, we can examine all the events of neural differentiation, from axon outgrowth to target exploration to the maturation of a synapse within the time constraints of a single shuttle flight. Our goals are to examine quantitatively four key events in the development and maturation of synapses during embryonic and post-embryonic life. These will be characterized using digital optical microscopy, immunocytochemistry, and single cell morphometry. As development in the *Drosophila* embryo can be suspended and resumed by temperature shifts, it will be possible to accurately control the exposure to microgravity, and examine discrete developmental exposures covering critical times in the differentiation of the motoneurons. The morphological development of RP1 and RP3 will be determined: 1) as they navigate the embryonic CNS and periphery to seek out their peripheral targets; 2) as they innervate their respective muscle fibers; and 3) as the synapses differentiate and develop their mature form during embryonic and post-embryonic life. Finally, 4) we will determine the extent to which the neurons and their targets maintain correct connectivity during development under the conditions of space flight. We propose that by focusing on

two singly identified neurons with already well understood normal development, any developmental errors involving axon guidance and synaptogenesis will be readily detected and interpreted.

General goals and accomplishments: The project being performed for Neurolab has moved forward excellently during the last year. We now have two lines of flies which we are ready to use for our designated flight of October 1998. Two key tasks remain to be solved prior to flight. First, we need to test the behavior of these animals in the designated hardware for the flight, namely the BRIC canisters maintained in CRIM incubators. Second, we need to finely calibrate the timing shifts for the experiment in light of the results from the temperature shift experiments performed with BRIC canisters. Both tasks seem readily accomplishable, and we are confident that we can complete necessary ground preparations for next year's flight opportunity.

Recent discussions with NASA Ames has led to the plan for them to do preliminary testing with BRIC canisters. Among our key concerns is the time it takes for petri dishes transferred between BRICs to equilibrate at a new temperature. This will directly affect the timing of the planned temperature shifts.

The major goal from the animal side, however, has been achieved, namely to develop *Drosophila* lines where we can assay individual neuromuscular endings directly without dissection. This was achieved by using the GAL4-UAS system, where we have succeeded in establishing stocks of flies where the key neuromuscular connections can be assayed directly in undissected larvae by means of the expression of endogenously fluorescent reporters in the specific motor endings. The green fluorescent protein (GFP) as a reporter allows scoring of neural anatomy en masse in whole mount using fluorescent microscopy without the need for either dissection or specific labeling. Two stocks have been developed. The first, which we developed first, uses the S65T mutant form, which has a dramatically brighter expression than the native protein. This animal will use GAL4 drivers with expression under the control of the elav gene, and which will ensure expression in all neurons of the embryo and larva. The second transgenic animal we have developed is of a novel kind, and makes use of dicistronic design, so that two copies of the protein will be expressed per insert. We have also developed a tricistronic form, but this has not yet been transformed into flies, and we do not imagine that this third line will be ready in time for the flight.

The new lines have extraordinary GFP expression, and allow for all analyses of motor endings in undissected, whole mount animals. This will vastly simplify our analysis of the data, and will easily increase our database dramatically. We previously feared that the rate-limiting step of this project would be our ability to handle all of the needed tissue processing upon the end of the project. We have reduced the project to nearly no tissue processing.

Background Information on GFP Reporter Constructs: There is no difficulty in obtaining excellent images from undissected embryos at the developmental stages when the mesoderm and nervous system are undergoing their differentiation. The problem is to identify the relevant cells conveniently, and to do so in whole mount after stage 17 of embryogenesis is a daunting task. As noted in the original proposal, we had planned to avoid dissection, as this will be a major rate-limiting step in the analysis of the embryos and larvae. A goal of the ground-based studies for Neurolab is to develop robust cellular reporters to make this possible in whole mount embryos and larvae. We now have the tools needed to image neurons in undissected animals and get high resolution images through the cuticle in larvae. This has been made possible by the development of GFP probes of the jellyfish *Aequorea victoria* (Chalfie et al., 1994; Wang and Hazelrigg, 1994; Heim et al., 1994; Marshall et al., 1995). GFP is intensely fluorescent and shows relatively little photoinactivation.

A route to create fluorescently marked precursors is the GAL4/UAS expression system developed by Brand and Perrimon (1993). This technique allows one to use a regulatory element of interest to drive expression of the transcriptional activator GAL4. GAL4, in turn, binds to UAS sequences fused to the coding region of a reporter of interest, driving expression. For our work, we used a *Drosophila* line where the regulatory UAS sequences have been fused to the coding region of GFP. We are currently focusing our efforts on a GAL4-elav driver with strong neuronal expression during embryonic and larval development. As a reporter we are using a UAS-(S65T) mutant form of GFP, as well as the double mutant distronic form we have developed.

This line is especially advantageous, because it gives excellent whole animal expression in larvae. Using it we have succeeded in examining fluorescently neuromuscular projections as late as the third instar in undissected live animals. All central and peripheral neurons are intensely fluorescent, and they can be examined *in situ* through the cuticle.

Drosophila strains expressing GFP in neuromuscular endings greatly simplify anatomical screens to identify neuromuscular innervation phenotypes caused by hypoactivity regimes such as microgravity. In effect, synapses can now be considered to be externally visible structures. In larvae of the C155 -GAL4/UAS-GFP stock, all of the motor endings of the SNb nerve can be clearly imaged through the cuticle in live larvae. We have found that it is possible to line up larvae on a compound fluorescent microscope using low power optics (16X 0.5NA neofluars) to obtain excellent detail of the motor endings, including the presence of the appropriate motor ending arbor types (types Ib, Is, and II), and the branching patterns on the muscle fibers, as well as the presence of ectopic motor endings. These can be done both on an upright or on an inverted fluorescence microscope (either available for this project). The larvae examined in this fashion are unharmed, and will develop to adults. This makes it possible to perform screens for the effects of microgravity on synaptic structures.

Polycistronic GFP Expression Systems: This is being carried out by Dr. Marc Halfon of the lab. The new constructs represent a "third generation" GFP line, where we have made the double mutant GFP that is approximately 6-fold brighter than the S65T single mutant form. In addition, by employing the IRES system, we will be able to express multiple copies of GFP with a single promoter. We have created four vectors to date, and have successfully done transformations with three of them. The first was a proof of concept, where a metallothionein promoter was used to drive GFP expression in single and dicistronic forms in S2 culture cells. Fluorescence expression following induction was examined using a FACS cell sorter. The dicistronic form yielded cells with an approximately two-fold enhancement of expression over the conventionally transformed cell lines. In addition we created a UAS-diGFP dicistronic vector and successfully obtained *Drosophila* transformants. The transformed flies express UAS-GFP with a single copy insertion at roughly the same fluorescence and homozygotes with the conventional form. We are now preparing to inject for transformation a tricistronic form. When homozygosed, this fly will express six copies of GFP, each in a mutant form with 6-fold brighter fluorescence than the S65T forms we currently are using. We do not expect that these flies will be ready for ground testing in time for the flight. Nevertheless, they will be of outstanding value for future missions using *Drosophila*.

Benefits to space life science research: Studies on *Drosophila* have already demonstrated that it is an excellent model system for studying synaptogenesis at the cellular and molecular level. If plans exist for long-term human exposure to reduced gravity, it is essential that all consequences to normal development and plasticity be understood at the cellular and molecular level. Vertebrate somatosensory and motor systems undergo extensive plasticity throughout life (including the adult), and therefore microgravity may potentially cause long-term changes or injury to the CNS and peripheral synapses of humans. If prolonged exposure to microgravity is anticipated (as in the case of the space station or related missions), then these studies using a model genetic system will prove valuable for identifying the kinds of changes in nervous system connectivity which may occur in humans.

General benefits: Two general benefits will result from these studies: 1) The reporter constructs will be of great value to all researchers interested in examining nervous system development in *Drosophila*, both for mutagenesis studies and for examining normal development. Thus, the *Drosophila* lines being developed specifically for the Neurolab mission will be of wide utility to the research community for other studies. 2) Insights into the role of alterations in neuromuscular activity will be of considerable value in examining the problem of synaptic plasticity.

FY97 Publications, Presentations, and Other Accomplishments:

Chang, T.N. and H. Keshishian "Laser ablation of *Drosophila* embryonic motoneurons causes ectopic innervation of target muscle fibers." *J. Neurosci.*, 16, 5715-5726 (1996).

Chiba, A. and H. Keshishian "Neural pathfinding and recognition." *Develop. Biol.*, 180, 424-432 (1996).

Fernandes, J.J. and H. Keshishian "Patterning the dorsal longitudinal flight muscles of *Drosophila*: Insights from ablation of larval scaffolds." *Development*, 122, 3755-3763 (1996).

Halfon, M.S., Kose, H., Chiba, A., and Keshishian, H. "Targeted gene expression without a tissue-specific promoter: Creating mosaic embryos using laser-induced single-cell heat shock." *Proc. Nat. Acad. Sci.*, 94, 6255-6260 (1997).

Neuronal Development Under Conditions of Space Flight

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Funding:

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Students Funded Under Research: 0
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Solicitation: 93-OLMSA-01
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Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9301123
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center
Flight Hardware Required: RAHF

Task Description:

The proper development of the nervous system requires sensory input. For example, the development of sight requires visual input during a critical period. Children whose eye muscles are not properly aligned, a common condition called strabismus, tend to suppress vision in one eye. If the one waits beyond the critical period, even after the eyes are re-aligned, vision may not be restored. Visual input during the critical period is required for a person to see normally. This study is designed to determine whether the sensory information provided by gravity after birth is necessary for the development of spatial ability. Our first step toward answering this question will be to study the structure and function of brain areas, particularly the hippocampus, involved in spatial memory. The number of synapses will be counted in rats returning from space and compared to ground-based controls. The expression of certain key molecules that appear in the mature brain will be measured. We will determine whether several neurotransmitter systems, cytoskeletal proteins, and synaptic proteins are altered in their distribution. Learning how the brain handles gravitational cues allows us to begin to assess the feasibility of long-term habitation in space.

After extensive analyses of tissue from the flight simulations, we have devised a superior and simplified dissection of the tissue. The new method simply involves a brain bisection and we have verified that the tissue quality for immunocytochemistry and electron microscopy is superb. We have developed a standardized means to collect all of the serially sectioned hippocampal tissue for immunocytochemistry. We have also identified those protein markers that undergo significant developmental changes during the time period when the animals will be in flight. Specifically, the glutamate receptor subtypes show significant changes in expression and distribution within the developmental window we are studying. Using confocal microscopy, we have developed methods for quantitating these images.

An enhanced understanding of early brain development is crucial to providing infants and children an environment which allows the brain to attain its maximum capacity. This project will provide insights into

early brain development. Applications to current pressing medical conditions are also expected because spatial ability is frequently affected in a variety of brain diseases, including Alzheimer's disease and stroke.

Ensemble Neural Coding of Place and Direction in Zero-G

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Funding:

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Students Funded Under Research: 3 Post-Doctoral Associates: 2
FY 1997 Funding: \$397,562
Joint Agency Participation: NIH and Office of Naval Research

Flight Information:

Experiment ID: 9301100
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center

Task Description:

Recent neurophysiological and behavioral experiments strongly suggest that the capacity for rapid and effective spatial orientation is based primarily on the interaction between a set of high-order neurons that transmit a representation of spatial location and an extensive network of neocortical and subcortical neurons which use vestibular, angular velocity information to compute and transmit a signal reflecting the azimuthal component of the animal's head orientation relative to an inertial reference framework. Clearly, the fact that this orientation system is based on azimuthal information with respect to the local gravitational field suggests that problems may develop in low or zero-gravity situations. The present proposal for the Neurolab mission aims to use neurophysiological experiments in freely behaving rodents to address the question of how this crucial system performs and adapts under low gravity conditions. Methods developed in this laboratory have enabled the simultaneous recording from large numbers of neurons involved in the spatial orientation system and which enable the same neuronal ensembles to be studied over periods of up to several weeks. This technology will maximize the amount of relevant neurophysiological data that can be obtained from a small number of rodents (2-4). These rodents will be tested extensively before, during, and after space flight in a series of experiments designed to elucidate how the brain's navigation system is disrupted in microgravity, how it may adapt to these conditions over time, and how it may readapt to the 1-G environment on Earth after 16 days of space flight.

The Neurolab Experiment Verification Test was completed in the Fall of 1996. During this test we verified the logistics of running our experiment in a realistic flight scenario. Twenty animals were trained on the preflight tasks from L-52 to L-30. Fifteen of these animals were surgically implanted with neuronal recording devices at L-30. These animals were then further trained, and the recording electrodes were adjusted daily to optimize neuronal recording quality. At L-1, five animals were loaded into RAHF cages for the mission simulation. Simulated recording sessions were performed on FD4 and FD9 to verify the recording protocol. The Data

Acquisition System hardware and software design and testing were completed during this year. Two prototype systems have been built and tested. Four KC-135 flights were performed in July to test the Data Acquisition System and to verify the feasibility of neuronal recording in microgravity. Both objectives were satisfied. A Facilities Trial Run at Kennedy Space Center Hangar L was performed in August. The surgical, animal training, and neurophysiological recording laboratories were evaluated and were deemed suitable for the experiment. Tests were conducted to verify that the animals implanted with recording devices could access food and water in the Animal Enclosure Module (AEM), and that no damage to the recording devices resulted from being housed in the AEM. A number of Crew Training activities occurred both at the University of Arizona and at NASA facilities. These activities concentrated on familiarizing the crew with the aims of the experiment and the nature of neurophysiological recordings and behavioral paradigms.

The research seeks answers to fundamental questions about the brain mechanisms for the development of high-level cognitive maps of the world. The same neural structures are also involved in the establishment of long-term 'episodic' memories of experience. The advancements in neuronal recording technology made during this project will give researchers powerful new tools to understand how the brain works. Discoveries made with these techniques may help in learning not only how the brain functions normally, but also what goes wrong in pathological conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, and other brain disorders. For example, the structure studied in this experiment, the hippocampus, is one of the first brain areas affected by Alzheimer's disease. Our Neurolab experiment holds great promise in helping us to understand how this part of the brain works under abnormal conditions as well as normal conditions, and may lead to greater insight into the mechanisms that cause the brain to malfunction in Alzheimer's patients.

Reduced Gravity: Effects in the Developing Nervous System

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Funding:

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Students Funded Under Research: 2 Post-Doctoral Associates: 1
FY 1997 Funding: \$91,117

Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Experiment ID: 9301093
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center

Task Description:

This research will examine the short-term and intermediate-term effects of space flight and reduced gravity on the cells of the developing central nervous system (CNS). The objective of these studies will be to determine the effects on: 1) cell proliferation (i.e., possible changes in the number of proliferating cells or in the number of cells produced and in the length of the cell cycle and of the S-phase of the proliferating cells); and 2) neuronal migration (i.e., the rate of movement and attainment of proper position). For these studies, the focus will be on the development of the cerebral cortex, a well-studied structure for which there is a great deal known about normal development. Experiments will be performed on mice for which there already is a great deal of data from other NIH supported projects. For this analysis, two markers of cell proliferation, bromodeoxyuridine, which is detected immunohistochemically, and tritiated thymidine, which is detected autoradiographically, will be used. The two markers will be administered to pregnant mice during orbital operations at selected days during the development of the cerebral cortex. The short-term effects will be assessed by administering these markers and sacrificing the fetuses 2.5 hours later (after removal by caesarean section). The intermediate-term effects will be assessed by administering these markers and sacrificing after 1 to 3 day survival. For short-term studies, changes in the number of proliferating cells, the length of the cell cycle, and the length of the S-phase of the cell cycle will be determined at different ages and after different periods of time in space. For intermediate-term studies, the migratory fate of cells "born" at particular ages will be determined. Crucial to the interpretation of these studies is the fact that the experimental subjects are mouse fetuses which are insulated from the direct behavioral effects of space flight by their mothers and by the fact that their own brain is too mature to sense such changes in the environment.

Progress in FY97 has revolved around: 1) modifications of our established protocols in order for them to be successfully applied in the space shuttle, 2) the training of the crew, 3) continued development of our mathematical models and computer simulations, and 4) the collection of basic science data as well as preliminary

and control data in the strains of mice and rats to be used on Neurolab. All of these activities are being completed on time, and we are on schedule for the development of the project.

In the past year, we have completed several studies at NASA Ames Research Center (ARC) request with regard to compatibility of fixatives and solutions between our science needs and flight conditions. These have focused on storage conditions, i.e., time and temperature tolerances, etc. In addition, we have completed the development and testing of specific criteria to optimize the selection of pregnant mice for flight. The criteria that we have developed enrich the proportion of pregnant mice from the 50-60% proportion as received from the vendor to approximately 90-95%. This procedure is necessary because: 1) the vaginal plug screening procedure used by the vendor results in a pregnancy rate of only about 50-65%, and 2) there is no simple test of positive pregnancy in mice (or other rodents) because mice do not produce chorionic gonadotrophins. The necessity to select the maximum number of pregnant animals per group is required for the success of the experiment, i.e., an on-orbit non-pregnant animal will produce 0% science return. The selection procedures developed by the PI and Co-PI increase the pregnancy rate to over 90% by the stage of pregnancy needed for cage-load (i.e., one day prior to launch). With 3 animals per experimental subgroup selected for flight, a 90% pregnancy rate means that the probability of 3 non-pregnant animals being in a single subgroup is $(1-0.9)^3 = 0.001$. Furthermore, with 6 experimental subgroups the probability that none of the subgroups will consist of 3 non-pregnant animals is $(1-0.001)^6 = 0.994$. Thus, this enrichment procedure for the preflight selection criteria are designed to ensure a >99% change of achieving information from all 6 experimental subgroups and assure that the flight experiments will be successful.

The "Experimental Verification Test" (EVT) for the Neurolab flight was performed in October-November, 1996, at ARC. This included a complete dry-run of this experiment and also of the other projects for the Ames portion of the Neurolab flight. EVT itself went well and all portions of the planned experiments were completed. Tissue quality from EVT was excellent and demonstrated that tissue collection under simulated flight conditions is possible. The post-EVT period was used to modify and improve tissue flow, to modify and improve the instructions to be provided to the crew in flight, etc.

The "Facilities Test Run" (FTR) for the Neurolab flight was performed in August, 1997, at KSC. For E093, the FTR was an important test of three aspects of the experiment. First, it was a test of the possible impact of the microbiological barriers in the animal facility of Hanger L at KSC on the experimental design of the mouse portions of our experiment. Second, it was a chance to evaluate our preflight selection algorithm under the same conditions and in the same facilities that will be used for flight in 1998. Third, we were able to evaluate the "fit" of our experiment requirements with the physical layout of Hanger L's laboratories. In all three aspects, the FTR was an unqualified success. Minor modifications in procedures resulted and the preflight selection algorithm was successful in selecting a high proportion (>90%) of pregnant animals for flight and ground control groups.

Crew training sessions were held in the PI's lab and at ARC. The Co-PI participated in these sessions and trained the crew to perform the experiment and to collect and fix the tissues for this experiment. These sessions were successful.

The Earth-based basic science data collection and the concomitant development of mathematical models and computer simulation portion of the project have been proceeding extremely well. We have had several publications (listed below) that have been supported in part by these funds. The basic data and the mathematical models will enable the data obtained in flight, from ground controls, and from hypergravity experiments to be interpreted in a quantitatively precise conceptual framework. In addition, the papers provide direct and tangible evidence of the significance beyond the space benefits of this project.

The effects of space flight on the developing CNS are essentially unknown. Our "null hypothesis" is that microgravity will have a profound effect on cell proliferation because of the loss of bouyancy of the organelles which will disrupt intercellular mechanisms associated with cytoskeleton and with energy utilization required during mitosis. This is of general significance to space flight because cell proliferation also occurs in adults,

including humans, chiefly in the skin, gut, and immune systems. It is also of relevance to wound healing, etc., both in space and on Earth. The data to be collected will provide specific and new insight into the complex cellular processes associated with cell proliferation and the intracellular mechanisms that regulate and control this process. Since these events occur on Earth in every multicellular organism, these experiments are of general relevance to an understanding of the basic biological process of cell proliferations. Thus, the results that we obtain will be of significance in understanding the normal controls on the regulation of cell proliferation and cell number during development, in cancer, in immune system function, in wound healing, etc.

We plan to continue our studies of normal development and the development of our mathematical models and computer simulations. These studies are of significant value in their own right and also serve to maximize the scientific return and interpretability of the animals from the Neurolab flight.

FY97 Publications, Presentations, and Other Accomplishments:

Cai, L., Hayes, N.L., and Nowakowski, R.S. "Clone size during neocortical development: Agreement of experiment and prediction." (Abstract) Soc. Neurosci. Abstr., 22, 524 (1996).

Cai, L., Hayes, N.L., and Nowakowski, R.S. "Homogeneity of rate of passage through the cell cycle in developing cortex." J. Neurosci., 17, 2088-2100 (1997).

Cai, L., Hayes, N.L., and Nowakowski, R.S. "Synchrony of clonal cell proliferation and contiguity of clonally related cells: Production of mosaicism in the ventricular zone of developing mouse neocortex." J. Neurosci., 17, 2079-2087 (1997).

Chetverukhin, V.K., Chernigovskaya, E.V., Danilova, O.A., and Nowakowski, R.S. "Sex differences in the cell cycle parameters of the proliferative ventricular epithelium (PVE) in the developing rat preoptic area." (Abstract) Soc. Neurosci. Abstr., 22, 525 (1996).

Chetverukhin, V.K., Chernigovskaya, E.V., Danilova, O.A., and Nowakowski, R.S. "Sex-related differences in the cytokinetic parameters of the proliferative ventricular epithelium (PVE) in the developing rat preoptic area." International Endocrinology Society Meeting in St. Petersburg, Russia (1996).

Egger, M.D., Nowakowski, R.S., Peng, B., and Wyman, R.J. "Patterns of connectivity in a *Drosophila* nerve." J. Comp. Neurol., 387, 63-72 (1997).

Hayes, N.L., Fu, D., Reuhl, K.R., and Nowakowski, R.S. "Low doses of methylmercury affect cell cycle progression in developing cortex." (Abstract) Soc. Neurosci. Abstr., 22, 981 (1996).

McAuliffe, W.G. and Nowakowski, R.S. "Microglia in the striatum hypertrophy and proliferate after a single dose of MPTP." (Abstract) Soc. Neurosci. Abstr., 22, 913 (1996).

Miyama, S., Takahashi, T., Nowakowski, R.S., and Caviness Jr., V.S. "A gradient in the duration of the G1 phase in the murine neocortical proliferative epithelium." Cerebral Cortex, 7, 678-689 (1997).

Nowakowski, R.S. "Early development of the nervous system: cell proliferation and neuronal migration" in "Pediatrics and Perinatology, Section 25. Development of the Central Nervous System." Edited by: P.D. Gluckman and M.A. Heymann Edward Arnold Publishers, London, 2nd edition, pp 359-364 (1996).

Nowakowski, R.S., Cai, L., and Hayes, N.L. "Regional differences in composition of retrovirally labeled clones during early development of the mouse neocortex." (Abstract) Soc. Neurosci. Abstr., 22, 524 (1996).

Takahashi, T., Goto, T., Miyama, S., and Nowakowski, R.S. "Intracortical distribution of a cohort of cells arising in the PVE." (Abstract) Soc. Neurosci. Abstr., 22, 284 (1996).

Takahashi, T., Nowakowski, R.S., and Caviness Jr., V.S. "Interkinetic and migratory behavior of a cohort of neocortical neurons arising in the early embryonic murine cerebral wall." *J. Neurosci.*, 16, 5762-5776 (1996).

Takahashi, T., Nowakowski, R.S., and Caviness Jr., V.S. "The leaving or Q fraction of the murine cerebral proliferative epithelium: A general model of neocortical neuronogenesis." *J. Neurosci.*, 16, 6183-6196 (1996).

Takahashi, T., Nowakowski, R.S., and Caviness Jr., V.S. "The mathematics of neocortical neuronogenesis." *Dev. Neurosci.*, 19, 17-22 (1997).

Role of Visual Cues in Spatial Orientation

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Funding:

UPN/Project Identification: 106-30 (E136)
 Initial Funding Date: 1996
 Students Funded Under Research: 3
 FY 1997 Funding: \$403,574

Solicitation: 93-OLMSA-01
 Expiration: 1999
 Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9301136
 Flight Assignment: Neurolab (STS-90, April 1998)
 Responsible NASA Center: Johnson Space Center
 Flight Hardware Required: NASA Virtual Environment Generator

Task Description:

The goal of this Neurolab experiment is to better understand how humans transform spatial orientation cues from egocentric to exocentric frames of reference, so as to perceive linear and angular orientation ("tilt," "location," "direction") and linear and angular motion ("speed" and "rotation"). On Earth, gravity provides an omnipresent cue which anchors our exocentric reference frame. Perceived self-tilt influences how we recognize objects around us and judge their angular orientation and shape. Conversely, the tilt, direction, motion, and shape of objects influence our own self-tilt, -direction, and -rotation. Perception of self orientation and object orientation are thus interdependent. In orbit, as we move in three dimensions, to what extent are we able to maintain a consistent exocentric reference frame? Does our ability to recognize object orientation and shape depend on this? How does the orientation, shape, and motion of objects around us influence self-orientation? What is the influence of haptic cues and otolith unweighting?

Astronauts often experience striking, labile "visual reorientation illusions" (VRIs) and more persistent "inversion illusions." These illusions create a variety of human factors problems, and can trigger vomiting. That they are so common indicates that ego-/exocentric sensory transformations are strongly affected by 0-G. We believe it is scientifically and operationally important to study them in orbit using quantitative methods. For similar reasons, we predict that 0-G will also strongly influence angular and linear self-motion perception. We predict that the recognition, orientation, and shape of visual objects will depend on the orientation of the exocentric frame of reference adopted by the observer. Our past research in 0-G has dealt only with self-tilt and -rotation created by a homogeneous field of random dots rotating about a frontal axis. Results showed astronauts become more dependent on visual and haptic cues.

This Neurolab experiment is composed of three experiments based on existing 1-G paradigms. The paradigms are designed to better define ego-/exocentric sensory transformations in 0-G, to understand how exocentric frame of reference affects recognition of visual object, and to define how altered CNS gravireceptor cue weighting influences the onset of visually induced linear motion sensation. Pre- and post-flight controls are required. The tests measure: 1) the influence of scene symmetry, scene rotation, orientation expectation, and haptic cues on self-tilt; 2) the effect of perceived orientation on visual object recognition and shape perception; and 3) the onset of x-axis illusory linear self-motion ("looming linearvection") with and without haptic cues. Experiments 1 and 3 require the NASA Virtual Environment Generator workstation or alternative helmet-mounted display to present controlled visual scenes to both free-floating and restrained astronauts.

Milestones during this final year of experiment development for the April, 1998 Neurolab mission have included:

- 1) Design, development, and delivery of a flexible "Experiment Manager" experiment control software package for the NASA Virtual Environment Generator (VEG). The Experiment Manager is controlled by a "Session Manager" developed by Lockheed Martin Engineering Systems (LMES). It sequences the scenes rendered by World Tool Kit (Sense8 Corp) and the VEG's dual Intergraph Z-13 graphics accelerators, and calculates results which are stored on disk, and passed to the "Downlink Manager," also developed by LMES.
- 2) Development of "virtual Neurolab" test scenes, using digitized photos of the JSC Neurolab crew training module interior.
- 3) Pilot testing of our flight and ground experimental protocols, once some components of VEG hardware became available in June. A subset of the full protocol was evaluated in brief periods of weightless parabolic flight aboard NASA's KC-135 aircraft in July. Subjects experienced VRIs using virtual room test scenes in parabolic flight. Sensations were markedly different from those obtained in similar circumstances using a randomly dotted "rotating dome" (Young, et al, 1986), which lacked architectural features.
- 4) Development of crew training materials and flight procedures. Crew training sessions in Boston (December, 1996) and Houston (April, July, and September 1997) using the VEG, and in Toronto using the a real "tumbling room" at ISTS (April). Crew training in parabolic flight using the VEG and flight protocols (September, 1997).
- 5) The VEG optical head tracker did not meet performance expectations, so protocols were modified so that it is no longer used in the experiment.
- 6) Delivery of final Experiment Manager scenes and scripts.

Many people are familiar with the illusions of visually induced self-tilt, circular-vector, and linear-vector through personal experiences in IMAX and "Circle Vision" theaters, amusement park rides (e.g., Disney's "Star Tours" and Universal's "Back to the Future"), or new "virtual reality" entertainment systems. There is currently considerable interest in using helmet-mounted "virtual reality" display techniques in a wide variety of applications in surgery, architecture, arts, education, manufacturing, mining, etc. Results from our Neurolab studies of interaction between visual, vestibular, and proprioceptive orientation cues in zero-G are generically applicable to the design of night simulator and "virtual reality" vision, motion, and cueing systems. Users of many existing systems report difficulty maintaining a consistent spatial frame of reference and motion sickness, because insufficient attention has been paid to providing appropriately matched visual, vestibular, and proprioceptive orientation cues. During the coming year, a collaborative program of ground-based research on human orientation in real and virtual environments is being initiated in our laboratories at MIT and York University with the support of the new National Space Biomedical Research Institute.

The vertebrate nervous system evolved in an environment where the stimulus to the various vestibular and proprioceptive gravireceptors invariably changed whenever the orientation of the body was altered. The unique weightless environment of orbital flight allows us to experimentally separate the visual, vestibular, and

proprioceptive cues of orientation, and thus better understand the role of gravity in the fundamental sensory, motor, and cognitive mechanisms which normally subservise spatial orientation on Earth. These are the mechanisms which allow us to stand and move about actively in the environment, all the while maintaining the sense of place and direction and the stability of the visual world. The investigators only become aware of these functions when they are compromised by inner ear or central nervous system disease. If this happens, our everyday lives are profoundly affected. Unfortunately, more than 90 million Americans suffer from some type of balance disorder. Patients with inner ear disorders often have difficulty walking at night or in crowded places, cannot see clearly, particularly when moving, cannot safely drive, and sometimes suffer incapacitating bouts of vertigo and nausea and injurious falls. Humans with hippocampal lesions or Alzheimer's disease show impairments on a wide variety of spatial and navigational tasks. There is much research interest in development of new methods for evaluating a patient's ability to use visual and proprioceptive cues in maintaining balance and orientation, and for improving balance function via rehabilitative training. Portable head-mounted displays, akin to those used in this Neurolab experiment, may well prove useful for such testing and rehabilitating, and perhaps someday even as visual prostheses for vestibularly impaired patients.

FY97 Publications, Presentations, and Other Accomplishments:

Oman, C.M. "Neurovestibular research in microgravity." (Poster) CNES Workshop on Neurosciences Research in Space, Paris, France (April 22 - 24, 1997).

Oman, C.M. and Skwersky, A. "Effect of scene polarity and head orientation on illusions in a tumbling virtual environment." (Abstract) *Aviat. Space Environ. Med.*, vol. 68, no. 7, 649 (1997).

Oman, C.M., Howard, I.P., Carpenter-Smith, T., and Beall, A.C. "Neurolab experiments on the role of visual cues in microgravity spatial orientation." 12th Man In Space Symposium: The Future of Humans in Space, Washington, DC (June 8 - 13, 1997).

Skwersky, A. Effect of scene polarity and head orientation on roll illusions in a virtual environment. (Thesis) Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA (August, 1997).

Effects of Microgravity on Neuromuscular Development

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Funding:

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Solicitation: 93-OLMSA-01

Initial Funding Date: 1994

Expiration: not available

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$112,947

Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Experiment ID: 9301122

Flight Assignment: Neurolab (STS-90, April 1998)

Responsible NASA Center: Ames Research Center

Task Description:

Space flight and hindlimb suspension unloading studies indicate that weightbearing may be required for normal development of the motor systems of land animals. Our long term goal is to understand the influence of microgravity on the development, maturation, and maintenance of the neuromuscular system of terrestrial mammals including humans. The proposed studies of rats will explore the hypothesis that gravity-associated weightbearing is required postnatally for normal neuromuscular development of motoneurons, neuromuscular junctions, and muscle fiber types of the antigravity soleus muscle, but not for that of the extensor digitorum longus (EDL), a nonweightbearing muscle. Rat pups (8 days old) will be exposed to microgravity for 16 days. Parallel groups of ground controls will be conducted on normal and hindlimb suspended unloaded (HSU) rats. This will generate baseline data on the effects of suspension unloading on the development of the neuromuscular system. Comparison of these findings with flight results will verify the fidelity of the suspension model for simulating microgravity effects on neuromuscular development. Space flight is expected to cause persistence of neonatal attributes and/or the development of anomalies in the soleus, but not in the EDL, and returning animals to terrestrial gravity is not predicted to reverse completely the aberrancies. These results will have strong implications for rearing normal animals, including humans, in the microgravity environment of space, and will further our understanding of the importance of weightbearing activity for motor system development of human infants on Earth.

Standard ground-laboratory procedures were adapted for the Neurolab mission, and new approaches were defined to facilitate on orbit processing of neonatal rat tissues. A tail gauge was constructed to measure tail length for the purpose of estimating body weight in microgravity in order to determine proper doses of anesthetics for survival and non-survival surgeries. A large number of postnatal rats at the Ames Research Center were weighed and tail length measured daily. The correlation of tail length and body weight was directly correlated at 95% confidence level. A nomogram was constructed providing the crew with a tail length/dose table for a Ketaset Cocktail

(ketamine, xylazine, acepromazine; im.) for survival surgery, and another table adding sodium pentobarbital ip. for non-survival surgery. This approach eliminated the need for a small mass measuring device on orbit.

A series of markers for retrograde labeling of soleus and EDL motoneurons were tested along with a Hamilton microsyringe injection apparatus for delivering 0.5 mm of marker into each muscle. Fluorescent latex beads (40 nm) provided an intense labeling in sections reacted for cytochrome oxidase histochemistry. With this approach, the identity of the labeled neuron was preserved, permitting direct evaluation of its oxidative enzyme properties. A skin incision was necessary to visualize the muscle to ensure correct muscle injection. Rapid, complete, and compatible closure was accomplished by replacing needle suturing with Nexaband, a tissue glue. The mother rat and the sibling did not bother the Nexaband-closed wounds.

Inflight perfusion of rats on flight days 8 and 15 is necessary for determining the effects of microgravity on neuromuscular development uncomplicated by the changes induced by the stresses of reentry and reloading in terrestrial gravity. The gravity flow whole animal perfusion procedure used on Earth was replaced by a Baxter InfusOR battery-powered pump. A ball-tipped needle was identified for self-anchoring the inflow in the left ventricle. These two modifications freed crew members' hands to perform additional tasks during perfusion. Perfusion was optimal when the PBS/sucrose solution was warmed to 37°C to cause vasodilatation and clearing of blood before introducing the fixative. A flight-approved solution warming bag was identified for this task. Crew proficiency and maintenance training in the above procedures was conducted. The KSC Hangar L laboratories were checked during a Facilities Trial Run and found satisfactory for accommodation of mission ground activities. Deficits in laboratory equipment and supplies were identified, and appropriate actions were taken to remedy the deficiencies.

Examination of neuromuscular development in microgravity is important for understanding the basic biology of nerve and muscle development and the role of gravity in development of humans on Earth. The neuromuscular system of the 8-day-old neonatal rat matures by 21 days which is comparable to the last 2 months *in utero* and first year of life for a human infant. Premature infants, living in incubators, are deprived of exercising their legs against the uterine wall, and infants may have diseases that limit normal weightbearing activity. To what degree compromised weightbearing delays or permanently alters normal neuromuscular development is unknown. The studies of neonatal rats will provide valuable insights into the role of gravity in the development process and if appropriate, may indicate exercise procedures to promote normal development in compromised infants. In addition, the testing of survival anesthesia, microsurgery, and vascular perfusion of rats in microgravity will improve our ability to treat injuries and deliver health care to humans during space flight.

FY97 Publications, Presentations, and Other Accomplishments:

Riley, D.A. "Is skeletal muscle ready for long spaceflight and return to gravity?." *Adv. Space Biol. Med.* (In Press).

Riley, D.A. "Review of primary spaceflight-induced and secondary reloading-induced changes in slow antigravity muscles of rats." *Adv. Space Res.* (In Press).

Vijayan, K., Thompson, J.L., and Riley, D.A. "Eccentric contraction-like damage occurs primarily in slow fibers of atrophic rat muscle." *Med. Sci. Sports Exer.*, 29, S65 (1997).

Widrick, J.J., Romatowski, J.F., Bain, J.L.W., Trappe, S.W., Trappe, T.A., Thompson, J.L., Costill, D.L., Riley, D.A., and Fitts, R.H. "Effect of 17 days of bed rest on peak isometric force and unloaded shortening velocity of human soleus fibers." *Am. J. Physiol.*, 273, C1690-C1699 (1997).

Flight Verification Test of Nursing Facility

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Funding:

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Solicitation: 93-OLMSA-01

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: not available

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R3 (STS-72, November 1995)

Responsible NASA Center: Ames Research Center

Task Description:

While pregnant rats and adult rats have been successfully flown in space, flying nursing neonatal rats and dams has not been attempted. Before proceeding with funding of Neurolab mammalian development studies, NIH has required NASA to demonstrate biocompatibility of a Nursing Facility (NF) cage with nursing neonatal rats and dams exposed to space flight and safely returned to Earth. For NIH.R3, six litters of 10 nursing neonates each, representing 3 age groups of neonates 5, 8, and 15 days old (PN5, PN8 and PN15, respectively) were flown for 9 days in Nursing Facility cages contained within 3 Animal Enclosure Modules (AEMs). Comparable animal numbers and ages were maintained in NF cages in operational AEMs on Earth. On landing day, we received one half of the neonates for assessment of animal health by video recording of movements and histological analysis of selected tissues. A portion of the neonates were permitted to recover for examination of long lasting effects of caging and space flight.

Rats launched at 8 and 15 days of age were permitted to recover in gravity for 9 months to assess whether permanent changes had been induced by 9 days of space flight-unloading during the neonatal period. The muscle fiber cytoplasm to myonucleus ratio was determined for single fibers from the soleus and EDL muscles of flight and ground control rats. Single fibers were isolated by collagenase digestion and fine mechanical teasing. Fibers were typed as either fast or slow based on the presence or absence, respectively, of fast myosin heavy chain immunofluorescence staining. Myonuclei were visualized by Nuclear Yellow hoescht dye staining. The cytoplasm:myonucleus ratio was measured using computer-assisted digitizing morphometry. The results show that the soleus and EDL muscle weight to body weight ratios were normal for flight rats. However, the cytoplasm:myonucleus ratio was increased only in the soleus muscle fibers of flight rats. These findings suggest that space flight-unloading permanently reduced the number of myonuclei in the weightbearing muscle.

The importance of gravity loading during muscle development is emphasized by the present results. A permanent deficit in the number of myonuclei occurred when weightbearing was eliminated by space flight. The mechanism remains to be elucidated. These findings indicate that loaded muscle contractions during mammalian

development, including humans, are necessary to achieve the normal complement of muscle nuclei. A deficiency in the number of nuclei potentially reduces the regenerative capacity and limits of the growth potential of the muscle. Problems may arise during aging because there is greater reliance on these factors at that time.

FY97 Publications, Presentations, and Other Accomplishments:

Huckstorf, B. Is hindlimb suspension unloading a high fidelity simulation of spaceflight effects on soleus and extensor digitorum longus development? (Medical Student Summer Research Report). Medical College of Wisconsin (1997).

Autonomic Neurophysiology in Microgravity

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Funding:

UPN/Project Identification: 106-30 (E095) Solicitation: 93-OLMSA-01
Initial Funding Date: 1996 Expiration: 1999
Students Funded Under Research: 7 Post-Doctoral Associates: 2
FY 1997 Funding: \$178,982
Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Experiment ID: 9301095
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Johnson Space Center

Task Description:

Alterations in autonomic nervous system function are likely responsible for many of the physiologic responses to space. Our overall objective is to determine in a definitive manner the effect of microgravity on the autonomic nervous system, combining physiologic, biochemical, and pharmacologic approaches.

In clinical protocols defined in ground-based studies and carried out in subjects studied preflight and during the Neurolab mission, we will assay plasma and urinary catecholamines and their metabolites, using HPLC with electrochemical detection, to define circulating levels of norepinephrine, epinephrine, and dopamine; their response to exercise; and their intra- and extra-neuronal metabolism. We will administer tracer doses of tritiated norepinephrine to assess norepinephrine spillover and determine whether alterations in clearance or release are responsible for the decreased plasma levels seen during space flight. We will directly measure sympathetic nerve traffic with microneurography and compare the responses of efferent sympathetic activity to physiologic stimuli such as skeletal muscle afferent stimulation with isometric and isotonic forearm exercise. Finally, we will define the effects of promethazine, commonly used to mitigate the space adaptation syndrome, on these parameters. These studies will provide a complete and definitive assessment of sympathetic function in space and will serve as a basis for subsequent studies of potential countermeasures.

Tasks entailing the development and implementation of study equipment and procedures reached near-completion during FY97. Preliminary testing of all techniques suggests that the information being sought in the Neurolab mission of 1998 should be forthcoming.

In ancillary studies, data have been developed that the syndrome of orthostatic intolerance in young women closely resemble the cardiovascular responses after space flight and appears to be due to partial dysautonomia.

The results of these studies will improve our understanding of autonomic mechanisms that regulate blood pressure. We hope that this will be translated in the development of improved countermeasures to alleviate the orthostatic symptoms astronauts experience upon return to Earth. It should be noted that orthostatic intolerance is the most common autonomic abnormality that affects a substantial number of patients. These patients are usually young and otherwise normal, but are significantly disabled by their inability to remain upright because of symptoms of cerebral hypoperfusion. This disorder is poorly understood; therefore, treatment remains inadequate. We believe the knowledge gained by the Neurolab experiments will help improve the treatment of these patients.

FY97 Publications, Presentations, and Other Accomplishments:

Costa, F., Davis, S.N., and Biaggioni, I. "Estimation of skeletal muscle interstitial adenosine in humans." (Abstract) *Drug Dev. Res.*, vol. 37, 189 (1996).

Feoktistov, I. and Biaggioni, I. "Prostanoid receptor with a novel pharmacological profile in human erythroleukemia cells." *Biochem. Pharmacol.* (In Press).

Feoktistov, I. and Biaggioni, I. "Role of adenosine in asthma." *Drug Dev. Res.*, vol. 39, 333-336 (1996).

Feoktistov, I., Sheller, J.R., and Biaggioni, I. "Adenosine A2b receptors in human lung cells as a target for antiasthmatic methylxanthines." (Abstract) *FASEB J.*, vol. 10, A1232 (1996).

Feoktistov, I., Sheller, J.R., Vallejo, V., and Biaggioni, I. "Immunological identification of adenosine a2b receptors in human lung mast cells." (Abstract) *Drug Dev. Res.*, vol. 37, 146 (1996).

Feoktistov, I., Vallejo, V., and Biaggioni, I. "Adenosine a2b receptors: g-protein coupling and calcium signaling." (Abstract) *Drug Dev. Res.*, vol. 37, 121 (1996).

Jacob, G., Ertl, A.C., Shannon, J.R., Furlan, R., Robertson, R.M., and Robertson, D. "The effect of standing on neurohumoral responses and plasma volume in healthy subjects." *J. Appl. Physiol.* (In Press).

Jacob, G., Robertson, D., Mosqueda-Garcia, R., Ertl, A., Robertson, R.M., and Biaggioni, I. "Hypovolemia in syncope and orthostatic intolerance. Role of the renin-angiotensin system." *Am. J. Med.* (In Press).

Jordan, J., Shannon, J., and Robertson, D. "The physiological conundrum of hyperadrenergic orthostatic intolerance." *Chinese J. Physiol.*, vol. 40, 1-8 (1997).

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Multidisciplinary Studies of Neural Plasticity in Space

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Funding:

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Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: \$20,000	
Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke	

Flight Information:

Experiment ID: 9301085
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center

Task Description:

The proposed research is a coordinated study of gravity sensor neural plasticity induced by relatively long-term space flight. It will employ modern morphological, electrophysiological, and molecular biological methods to obtain data before, during, and after space flight, and advanced computer technologies to reconstruct main findings. The long-term objective is to achieve a better understanding of neural plasticity in otolith organs. The hypothesis to be tested is that the gravity sensor plasticity already observed in rats exposed to microgravity on the SLS-1 and SLS-2 missions conserves functionality by increasing hair cell synaptic efficacy, particularly in intrinsic local microcircuitry. The specific aims are: 1) to learn more about the functional implications of gravity sensor plasticity through correlated anatomical and physiological studies of adaptive responses to microgravity; 2) to answer the question of whether plasticity is an early phenomenon quickly induced upon insertion into microgravity; 3) to answer the question of whether otolith organ plasticity includes changes in otoconial mass; and 4) to correlate the anatomical and molecular findings through computer reconstructions that incorporate results of this research. The studies should also help answer the question whether readaptation to Earth is independent of, or correlated with, the length of time of exposure to altered gravity.

The principal question answered this year was whether payload specialist training had resulted in excellent dissections within the time required for tissue preservation. Consequently, much time was spent in training payload specialists in dissection procedures and in preparing the tissue for study by transmission electron microscopy. As a result of this effort, there is confidence that new information will be forthcoming from this space experiment. In particular, whether synaptic plasticity is a very early adaptive response will be learned because of the early inflight dissection. Late flight dissection results will be correlated with those obtained from SLS-2, which showed that synapses doubled in type II hair cells. Training Dr. Maguay Paupard from Dr. Zukin's laboratory in dissection procedures was also carried out. It appears from her research that immunocytochemical results will be best obtained from late inflight and postflight dissections. Based on prior

morphological findings, information should be obtained about possible glutamate receptor site changes at these times.

Microgravity provides an excellent tool to learn more about synaptic and neuronal plasticity (changes in structure/function) wherever they occur. This is because changes in synapses in space have been dramatic and they will, therefore, be more amenable to study by other approaches, such as immunocytochemical and electrophysiological, to determine their significance in causal, functional and behavioral terms. Thus, the research findings are fundamental to understanding mechanisms underlying plasticity changes occurring elsewhere that are related to learning and memory. The software developed for 3-D reconstruction of neurons and innervation patterns in gravity sensors has numerous ramifications. For example, it permits the wiring pattern of a simple neuronal system to be unraveled for scientific study and simulation. For the first time, the architecture of a sensory end organ will be known in detail and this information can be applied toward learning functionality. The same software is being used in the scientific study of other parts of the nervous system and in embryological studies through Space Act Agreements with more than 30 universities and Federal Agencies. The software also provides the basis for developing virtual environment scientific and clinical laboratories. For example, a virtual environment surgery project is underway that will prove useful in training surgeons and in practicing patient-specific surgery before working on a patient. A virtual surgery workstation is also of value to NASA for long-term space flights during which unforeseen medical problems may arise that require intervention beyond the immediate expertise of crew members on the space vehicle. Virtual laboratories will permit training before necessary intervention takes place. This work has led to a Cooperative Agreement with Stanford University to collaborate on establishing a National Center for Biocomputation that will bring together academicians, federal agencies, and industrial partners to help accelerate biocomputational studies of many kinds. At the same time, disturbances and diseases of organs of balance are common on Earth as exemplified by the frequency of motion sickness, a disorder affecting both young and old in the general population. A variation of this disorder, Space Adaptation Syndrome, affects astronauts although the causality is different in that exposure to the novel environment of microgravity rather than motion per se is at fault. The research that tries to uncover the basic mechanisms underlying Space Adaptation Syndrome will simultaneously help us to better understand possible mechanisms underlying motion sickness and other balance disorders on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Ross, M.D. "Morphological evidence for local microcircuits in vestibular maculae." *J. Comp. Neurol.*, 379, 333-346 (1997).

The Stress of Space Flight: Effects on Learning

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Funding:

UPN/Project Identification: 106-30 (E052)
Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$376,683

Solicitation: 93-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 930152
Flight Assignment: NIH-H (STS-95, 1998 [target])
Responsible NASA Center: Johnson Space Center

Task Description:

From lift-off to post-flight re-acclimation, space flight is clearly a tremendous stressor. In space, astronauts are required to perform complex physical and mental tasks, yet relatively little information has been gathered on how this unique stressor impacts on the basic components of learning and performance. We propose to study how prolonged exposure to microgravity affects nonassociative and associative learning.

Nonassociative learning will be assessed by measuring sensory reactivity (startle response) to sudden noise. Through the concomitant measure of heart rate spectrum (HRS) and eyelid electromyography (EMG), we will assess sensory reactivity to white noise stimuli of various intensities. Associative learning guides the allocation of neural resources and provides a framework for the acquisition of casual relations. Classical conditioning of the eyeblink response provides a convenient platform on which to observe the acquisition of these relations. We have proposed to study the effects of space flight and adaptation to microgravity on the acquisition of this conditioned response using a 2-tone discrimination paradigm. As with nonassociative learning, our goals in the present proposal are to expand our ground-based subject pool and to perform more extensive in-flight tests.

To the extent that space flight and prolonged exposure to microgravity represent stressful life events, we hypothesize that crew members will exhibit a persistent state of neuromuscular and autonomic sensitization. Further, it is hypothesized that humans exposed to space flight and prolonged exposure to microgravity will exhibit enhanced acquisition of a classically conditioned response.

Project staff worked with NASA subcontractors on hardware changes to the eyeblink conditioning equipment throughout FY 1997. A preliminary hardware Critical Design Review was held in Houston in February, 1997. Prototype hardware was delivered in August, 1997 and required further modification. Delivery of another prototype is expected in FY 1998. The Payload Integration Plan was reviewed in Houston in May of 1997 and

has been approved. The Institutional Human Subject review board approved the shuttle and KC135 flight protocols. The experiment is now tentatively scheduled for a shuttle flight in the fall of 1998.

These studies directly examine the interplay between environmental stressors and adaptation. Rarely does the scientist interested in the psychophysiological aspects of stressor exposure have the opportunity to measure human reactivity during a naturally occurring sequence of stressors. Moreover, the stressors of space flight and adaptation to microgravity have the potential of being more homogeneous in terms of intensity between individuals. Since stressor intensity is considered a critical variable in the genesis of stress-related mental illnesses (such as post-traumatic stress disorder), the results of these studies could indicate how stressor intensity contributes to these disease processes.

FY97 Publications, Presentations, and Other Accomplishments:

Shors, T.J., Mathew, P.R., and Chachich, M.E. "NMDA receptor antagonism in the basolateral but not central nucleus of the amygdala prevents the induction of facilitated learning in response to stress." Soc. Neurosci. Abstr. (In Press).

Tapp, W., Servatius, R., Hunt, J., and Powell, D.A. "Vagal activity predicts eyeblink conditioning in human subjects." NeuroReport, vol. 8, 1203-1207 (1997).

Effects of Microgravity on Postnatal Motor Development

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Funding:

UPN/Project Identification: 106-30-04 Solicitation: 93-OLMSA-01
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Students Funded Under Research: 2 Post-Doctoral Associates: 1
FY 1997 Funding: \$147,186
Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Experiment ID: 9301150
Flight Assignment: Neurolab (STS-90, April 1998)
Responsible NASA Center: Ames Research Center
Flight Hardware Required: Animal Walking Apparatus

Task Description:

The objective of this proposal is to evaluate the adaptability of the motor nervous system to environmental demands. The force of gravity is one of the few constant factors during the evolution of the nervous system and, for this reason, is deeply embedded in its functioning. This is particularly marked for the motor system since an animal's posture is dependent on the appropriate force being maintained at every joint of the articulated skeleton to oppose the action of gravity. The experiments examine the adaptability of the motor system to changes in gravity and the mechanisms underlying such neuronal plasticity. Since young animals are particularly susceptible to changes in their environment, they offer a sensitive model for nervous system plasticity. Our working hypothesis is that: 1) a normal gravitation field is essential for the normal postnatal development of the motor system; 2) elimination of weight-bearing will lead to profound changes in motor system organization; 3) changes in motor function will be most marked when animals are exposed to microgravity during "sensitive periods;" and 4) functional changes will persist into adulthood when animals are exposed during "critical periods" of motor development. We will use behavioral, electromyographic (EMG), and molecular approaches to study rat pups from postnatal day 6 (P6) through P31 in ground and flight studies. Behavioral measures will evaluate the development of interlimb coordination (e.g., swimming and walking), dynamic postural stability (e.g., placing reactions and righting reflexes), and complex motor skill (e.g., rope, ladder, and rod climbing). EMG recordings of activity from major hindlimb muscles will be combined with video-based motion analysis of treadmill walking to examine the neuronal basis for locomotion in control and experimental animals. Biochemical and immunohistological studies will determine the pattern of expression of glutamate receptor

subunits genes in the lumbar spinal cord. Pre-flight ground experiments will study animals reared under conditions of simulated microgravity (tail-suspension), hypergravity (centrifugation), and a simulated shuttle mission gravitation profile, hypergravity-microgravity-hypergravity. Rat pups will be reared aboard the shuttle P7-P21 (sensitive period) and P17-31 (critical period). In-flight experiments will evaluate placing reaction, complex motor skills, and vestibular reflexes. Post-flight, we will study the ability of the animals to adapt to the relative hypergravity of Earth. Neurolab offers a unique opportunity; the flight rats will be the first mammals to have developed the majority of their motor skills under conditions of microgravity. Study of these "space rats" will further our understanding of the role of gravity in postnatal development and to what extent the nervous system is able to adapt to changes in gravity. Since the major elements of the motor system—neurons, muscles, and bone—will develop under condition of microgravity, these experiments will also further our understanding of the plasticity and interaction of these systems during postnatal development and motor function.

In the last fiscal year, we have continued to analyze the flight NIH.R3 data, that from the experimental verification test data and to prepare for flight. We found that neonates can survive 2-G from P8-P24 and from P14 to P30. For the first three days at 2g they did not move around very much when the centrifuge was on. However, in the following, one could not tell if the centrifuge was spinning or quiet on the basis of the behavior of the animals. Behavioral tests were carried out at the end of the 16 days. Air righting times for the head, forelimb, and hindlimbs were not affected by hypergravity. This is in contrast to tail suspension and microgravity, where slow righting was seen. Swimming stroke duration was the same in hypergravity and environmental control animals. However, swimming style was distinctive in the hypergravity animals, the strokes being marked by extension of the hindlimbs compared to controls. This was quite similar to that seen in the flight and tail suspended animals. The most influence was seen in the gait of the hypergravity animals. They showed an extension of the ankle and knee angles during the stance phase compared to control animals. The knee angle showed the larger difference. This is in contrast to microgravity in which the ankle was more effective than the knee. These changes did not last as long as those seen after 9 days of microgravity, walking was similar to controls after 5 days in the younger animals and after 8 days of recovery in the older animals. We have also developed 35 probes for *in situ* hybridization studies of the spinal cord and established the optimal fixation and processing techniques for immunocytochemical and electron microscopic studies of brain and spinal cord tissue. Training has progressed with the in-flight aspects of the experiments.

The results of such a study will further our understanding of postnatal neuronal development, as changes in gravity provide an excellent noninvasive model for investigating nervous system plasticity. Mechanisms that underlie neuronal development are often the same that regulate plasticity and repair in the adult nervous system. For example, axotomized adult motoneurons show many properties of immature motoneurons; polyinnervation typical of the early postnatal period is seen after sciatic nerve block within adult motoneurons. Recently, it has been shown that activity-dependent synaptic plasticity in the adult and young animals follow the same general principles. Insights gained from space may be applicable to a number of neurologic conditions when plasticity of neuromuscular function would be desirable. For example, if reorganization within the nervous system could be enhanced by manipulating the glutamate receptor phenotype of neurons, enhanced motor function could result after trauma to nerve, muscle, or spinal cord, or in degenerative conditions of the neuromuscular system. Simulated weightless paradigms may also be relevant to pediatric cases where children are confined to bedrest.

FY97 Publications, Presentations, and Other Accomplishments:

Heffernan, C., Benavides, L., and Walton, K. "Effects of space flight on righting reflexes and swimming in neonatal rats." (Abstract) Soc. Neurosci., 23, 637 (1997).

Sulica, D. and Walton, K. "Walking in neonatal rats exposed to microgravity or hypergravity during postnatal development." (Abstract) Soc. Neurosci. 23, 637 (1997).

Sulica, D. and Walton, K. "Effects of a nine-day shuttle mission on the development of locomotion in neonatal rat." Houston Conference on Biomedical Engineering Research, 15:86 (1997).

Sulica, D., Heffernan, C., Benavides, L., Anshel, D., and Walton, K. "Effects of a nine-day shuttle mission on the development of the neonatal rat nervous system: a behavioral study." 12th Man in Space Symposium, 12th Man in Space Symposium Abstracts Volume, abstract pp. 274 (1997).

Walton, K. "Postnatal development under conditions of simulated weightlessness and space." Brain Res. Rev. (In Press).

Walton, K., Heffernan, C., Sulica, D., and Benavides, L. "Changes in gravity influence rat postnatal motor system development: From simulation to space flight." ASGSB Bull., 10, 111-118 (1997).

Flight Verification Test of Nursing Facility

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No Co-Is Assigned to this Task

Funding:

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 Students Funded Under Research: 0
 FY 1997 Funding: not available

Solicitation: 93-OLMSA-01
 Expiration: not available
 Post-Doctoral Associates: 0

Flight Information:

Flight Assignment: NIH-R3 (STS-72, November 1995)
 Responsible NASA Center: Ames Research Center

Task Description:

This is a feasibility study designed to: (1) determine if neonatal rats, when flown with their dams, will survive a 14-16 day space flight and their return to a 1-G environment, and (2) evaluate the health, neuromuscular status, and behavior of the surviving animals. The general health of the animals will be evaluated by a veterinarian from the Ames Research Center, the neuromuscular system will be evaluated by Dr. Danny Riley at the Medical College of Wisconsin. Behavioral measures will be carried out in our laboratory at NYU Medical Center. Litters with pups at postnatal day 4 (P4) and P8 at shuttle loading will be tested. Pre-flight experiments will determine the effect of simulated transport, take-off and landing, and, intermittent 0-G using KC-135 parabolic flights. There will be one control group; there will not be any simulated weightlessness controls.

These experiments are complementary to those of the ARC veterinarian and Dr. Danny Riley. Together, we will evaluate maternal behavior and pup development under three conditions: (1) Ground simulation of a space shuttle flight to include centrifuge simulation of take-up and landing G-force profiles, transportation, and RAHF cage housing. (2) Parabolic flight tests of the effect of alternating short periods of increased and decreased gravity in the type of cage to be used in the flight. (3) A two-week shuttle mission. Swimming speed will be evaluated in the same way in all three studies from G + 0 days through G + 4 days. Since this is a verification experiment, further data analysis of the centrifuge and parabolic flight animals will depend of the results of the initial data analysis. If we find no significant differences between experimental and control animals after flight, further data analysis and recovery points may not be needed. We do not expect to see delayed effects, but we will observe the general behavior of the animals for up to 30 days. Complete data acquisition and analysis will be carried out on flight animals.

A major effort during this project was devoted to test the ability of a rodent cage to support a nursing dams and neonates during a shuttle mission, NIH-R3. This effort included an opportunity to examine the behavior of the animals in microgravity and the effects of microgravity on the postnatal development of motor system function.

Survival of nursing rat neonates in microgravity. Six dams, each with a litter of 10 neonates were exposed to the conditions of microgravity for 9 days (1/11/96-1/20/96) during three developmental periods; P5 to P14, P8-P17, and P15 to P24 (2 litters at each age). Nineteen of the animals launched at P8 and all of the P15 animals survived the flight, while only 6 of those launched at P5 survived. Our role was to carry out behavioral measurements on the flight and control animals to find if the Mammalian Development Team Neurolab objectives could be met. We concluded that the Neurolab objectives could be met if animals are launched at P7 (age at the actual launch was P7, not P8) and P15, but not if animals are launched at P5.

Effect of microgravity on weight gain. Body weight was recorded on one flight and on flight cage housed ground control litter at each age from landing (R+0) until they were shipped to NYU on R+14. Animals launched on P5 (n = 3) and P8 (n = 10) weighed less than the ground controls throughout this period. (All control litters n = 10.) A significant difference persisted at R+60 in the P5 animals; however, the functional development of these animals was not retarded - they opened their eyes at the same age as ground controls, conceived, and gave birth to a litter within a week of the ground control animals. The litter was not distinguishable from others born in our laboratory. The P8 animals weighed 80% of the same-cage ground control animals, a significant difference, but not within the range used in studies of undernourishment. Although the animals were small, further studies would be needed to determine if they were undernourished. The P8 animals were similar to controls at R+60 days. There was no statistical difference between P15 (n = 10) flight and flight cage housed ground controls. Both groups were lighter than the vivarium-caged ground controls, however, indicating a "cage effect" probably due to crowding in the flight cages; this difference was no longer present at R+60 days. (A brief report was included in the NASA +30 days report, document number: AN-03006.)

Effect of microgravity on the development of motor function. Of the motor performance tests we have completed measuring swimming for R+0 to R+3, the flight animals swam slower than the AEM controls, but not slower when compared to a larger vivarium control group from our laboratory. Comparison of stick figures of the mean position of the hindlimb during a stroke in a control (left, n = 5) and flight animal (right, n = 5) on R+1 showed that the flight animal moved its limb further through the water before flexing it to enter the recovery phase of the stroke. Flight animals also tended to walk slower than the AEM animals. The most remarkable characteristic, however, was the extension of the joints during each step. During the stance phase, the ankle is more extended in the flight animal, but during the swing phase when the limb is lifted from the ground, there is no difference. Analysis of the mean ankle angle in several animals, shows that the difference seen in the figure is statistically significant at $p < .0001$. Flight animals also lift both the front and back leg higher off the ground than the control animal. The foot trajectory of the back foot was also elongated. It took the animals about two weeks to readapt to Earth gravity in most respects.

The results of such a study will further our understanding of postnatal neuronal development; changes in gravity provide an excellent non-invasive model for investigating nervous system plasticity. Mechanisms that underlie neuronal development are often the same that regulate plasticity and repair in the adult nervous system. For example, axotomized adult motoneurons show many properties of immature motoneurons; polyinnervation typical of the early postnatal period is seen after sciatic nerve block with in adult. Recently it has been shown that activity-dependent synaptic plasticity in the adult and young animals follow the same general principles. Insights gained from space may be applicable to a number of neurologic conditions when plasticity of neuromuscular function would be desirable. For example, if reorganization within the nervous system could be enhanced by manipulating the glutamate receptor phenotype of neurons, enhanced motor function could result after trauma to nerve, muscle, or spinal cord, or in degenerative conditions of the neuromuscular system. Simulated weightless paradigms may also be relevant to pediatric cases where children are confined to bed rest.

Sleep and Respiration in Microgravity

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$455,000

Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

Experiment ID: 9301198

Flight Assignment: Neurolab (STS-90, April 1998)

Responsible NASA Center: Johnson Space Center

Task Description:

There is evidence that sleep is affected by microgravity. However, despite anecdotal reports of poor quality of sleep in microgravity, and the common use of mild sedatives to improve the quality of sleep in the space shuttle, there have been no detailed studies of sleep in microgravity. In many people, nocturnal hypoventilation leads to hypoxemia and hypercapnia, and is a potent arousal stimulus. During sleep, many people experience periodic breathing, and there is one report of sleep apnea actually occurring in flight aboard the Russian Space Station Mir. Possible changes in the chemoreceptive control of ventilation brought about by exposure to microgravity may well contribute to alterations in the sleep pattern in microgravity.

We will measure respiration during sleep in microgravity by instrumenting subjects with a Respiratory Inductance Plethysmograph (RIP) and pulse oximeter allowing continuous measurement of the motion of both the rib cage and abdomen and arterial oxygen saturation. In addition, subjects will be fitted with an EEG, EOG, an ingestible body temperature sensor allowing us to determine sleep stage, and an ECG. From these sensors, we can determine changes in ventilation, relative rib cage and abdominal contribution to ventilation, thoraco-abdominal asynchrony, sympathetic and parasympathetic contributions to heart rate variability, and the coupling between respiration and heart rate, all as a function of sleep stage. There is strong evidence that there are neurological changes in the cardiovascular system brought on by exposure to microgravity, and we expect to find that there will also be changes in the neurological control of ventilation in microgravity. We expect that these will manifest themselves as changes in the pattern of sleep.

In addition, we will study the neurological control of ventilation by measuring the ventilators response to both hypoxia and hypercapnia. Inflight, we will measure the quasi-isocapnic hypoxia response and the hypercapnic rebreathing response. In addition, we will measure cardiac output, diffusing capacity lung water, and resting oxygen consumption. These will be supplemented by RIP and pulse-oximetry measurements allowing determination of respiratory timing without the interference of a mouthpiece and arterial oxygen saturation. Preflight and postflight, we will perform the same measurements and will, in addition, perform carefully controlled isocapnic hypoxic ventilatory response tests, as well as carotid baroreceptor-cardiac reflex. This will provide us information regarding the change in ventilatory control and the ventilatory-baroreceptor integrated reflex. The combination of the sleep studies and the awake measurements performed on the same subjects in microgravity will shed considerable light on the changes in the neurologic control of ventilation that occur when gravity is removed.

In FY 1997, we completed several hardware and software tasks. The new ALFE flight software was written, tested, and delivered. The ALFE flight hardware refurbishment was completed, and the new flight hardware for maintaining isocapnia during the hypoxic stimulation test was built and delivered. The digital sleep recorders were procured, extensively tested, and delivered. The status at the end of FY 1997 was that Level IV integration of rack hardware was complete and ready for test, the Science Verification Testing of the ALFE system was complete, and nominal crew training was complete. Crew malfunction training was ongoing. Crew procedures were essentially finalized and preparations for Baseline Data Collection were beginning. ALFE team members participated in two mission simulations during FY 1997. Successful completion of sleep data down link was demonstrated including the ability to effectively deal with partial file fragments in a timely fashion.

Sleep is often poor in microgravity and also in many terrestrial situations. This integrated study will examine the contribution of alterations of the control of ventilation to sleep disturbance, and also examine the usefulness of melatonin as an hypnotic agent. Both aspects have direct potential for benefiting sleep on Earth.

Development of Vestibular Organs in Microgravity

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Funding:

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Students Funded Under Research: 3

Post-Doctoral Associates: 2

FY 1997 Funding: \$ 165,543

Joint Agency Participation: National Science Foundation

Flight Information:

Experiment ID: 9301004

Flight Assignment: CEBAS (STS-89, January 1998) and Neurolab (STS-90, April 1998)

Responsible NASA Center: Ames Research Center

Task Description:

Little is known about the factors which control development of the vestibular system. One aspect that is likely to be affected by the gravitational field is the formation of the otoliths, the dense calcified masses upon which gravitational forces act, and their associated sensory structures. If the size of the otoliths is regulated on the basis of their weight, one would expect larger than normal masses to be produced in microgravity. The synaptic connections in the central nervous system responsible for otolith-mediated reflexes are also susceptible to unique factors when they develop in the absence of gravity. The work proposed here will address the formation of the gravity-sensing apparatus in two model systems which will undergo significant portions of their embryonic and larval development during the Neurolab mission. Adult and embryonic specimens at several developmental stages of the fresh-water snail *Biomphalaria glabrata* will be flown in the Closed Equilibrated Biological Aquatic System (CEBAS). After recovery, some specimens will be fixed for light and electron-microscopic examination. The statoconia in these specimens will be compared to ground-reared controls. Other specimens will continue to develop on Earth to test whether differences in statolith and statoconia production proceed at a normal rate after return to 1-G conditions. In the fish *Xiphophorus helleri*, the structure of the otoliths in ground-reared and space-reared animals will be compared at the light, electron-microscopic, and atomic-force microscopic level. As well as elucidating the effects of the microgravity conditions of the formation of these test masses, these studies will offer new insight to the control of otolith formation and maintenance. Recent studies indicate that demineralization of otoconia may contribute to balance problems in elderly humans, and knowledge of the mineralization process will aid in addressing this form of pathology.

The majority of our work in the last year has been directed at making preparations for the small-payloads flight on STS 89 (January, 1998) and Neurolab on STS 90 (April, 1998) of the CEBAS. Trial runs have been made

with the CEBAS minimodule in both Bochum, Germany, and two PVT runs at the Kennedy Space Center, run in July, August, and September, 1997. Our team has established procedures to work with German and other American investigators to retrieve samples from the CEBAS, including snail spawn packs laid during the test run, neonatal snails hatched during the run, juvenile and adult snails loaded into the system before simulated launch as well as embryonic, juvenile, and adult swordtail fish. Embryos are retrieved from the ovaries of the adult fish. Samples to be used for anatomical study were fixed and embedded either immediately after simulated landing or five days later, to assess the effects of this period on Earth after rearing in micro-G. We have also been able to videotape crawling patterns of snails from 1 to 7 mm diameter both on a vertical plate and on our specially constructed centrifuge, simulating 1- or 2-G conditions. The higher G condition will be used to test flight-reared snails in case they do not display the characteristic positive geotactic reflex exhibited by Earth-reared animals.

The fine structure and development of the statocyst of the snail, *Biomphalaria glabrata*, which will be flown in the CEBAS, have been investigated, as described in the two papers by Gao and Wiederhold, cited below. From these studies, we have established that the statoconia are formed in the supporting cells separating the statocyst receptor cells and exocytosed into the statocyst lumen and that they continue to add layers of calcification while they are in the lumen. Presumably, the supporting cells secrete additional proteins which facilitate additional mineralization of the statoconia after they are exocytosed. This offers a mechanism by which altered G conditions could affect statoconia production and maintenance.

It is well known that animals and man lose calcium from their bones during extended times in space. Our studies are designed to help understand what processes control biomineralization. There is growing evidence that the lack of gravity can adversely affect bone mineralization even in isolated embryonic bones. Thus, there appears to be a fundamental interaction between mineralization and gravitational forces. Such an interaction could have major consequences in a developing gravity-sensing organ which depends on the gravitation force on a dense calcified mass to activate sensory receptor cells. Our studies will address both the formation of the "test mass" in microgravity and the role of gravity during the development process in establishing gravity-related reflexes.

FY97 Publications, Presentations, and Other Accomplishments:

Gao, W., Wiederhold, M.L., and Hejl, R. "Development of the statocyst in the freshwater snail *Biomphalaria glabrata* (*Pulmonata*, *Basommatophora*)." *Hear. Res.*, 109, 125-134 (1997).

Gao, W., Wiederhold, M.L., and Hejl, R. "The structure of the statocyst of the freshwater snail *Biomphalaria glabrata* (*Pulmonata*, *Basommatophora*)." *Hear. Res.*, 109, 109-124 (1997).

Gao, W., Wiederhold, M.L., and Hejl, R. "Ultrastructure of the endolymphatic sac in the larva of the Japanese red-bellied newt *Cynops pyrrhogaster*." *Cell & Tiss. Res.* (In Press).

Gao, W.Y., Wiederhold, M.L., and Hejl, R.H. "The structure and development of the statocyst in the pond snail *Biomphalaria glabrata*." Abstracts of the Twentieth Midwinter Research Meeting of the Association for Research in Otolaryngology, 20:38 (1997).

Gao, W.Y., Wiederhold, M.L., and Hejl, R.J. "The structure and development of the statocyst in the pond snail *Biomphalaria glabrata*." (Poster) Twentieth Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, FL (February 2 - 6, 1997).

Pedrozo, H.A., Schwartz, Z., Dean, D.D., Harrison, J.L., Campbell, J.W., Wiederhold, M.L. and Boyan, B.D. "Evidence for the involvement of carbonic anhydrase and urease in calcium carbonate formation in the gravity-sensing organ of *Aplysia californica*." *Calc. Tiss. Int.*, 61, 247-255 (1997).

Pedrozo, H.A., Schwartz, Z., Dean, D.D., Wiederhold, M.L., and Boyan, B.D. "Regulation of statoconia mineralization in *Aplysia californica* *in vitro*." *Connect. Tiss. Res.*, 35(1-4), 317-323 (1996).

Wiederhold, M.L. "Development of gravity-sensing organs in microgravity." American Society for Gravitational and Space Biology. Oral Presentation, International Space Station Symposium. Twelfth Annual Meeting for the American Society for Gravitational and Space Biology. Radisson Plaza Hotel, Charlotte, North Carolina (October 24, 1996).

Wiederhold, M.L. "Development of gravity-sensing organs in the absence of gravity: Newts in space!" Oral Presentation, George F. Fried Seminar Series. Department of Biology, Brooklyn College of the City University of New York, NY (October 18, 1996).

Wiederhold, M.L. "Organ in microgravity." Oral Presentation, Fifteenth Annual Houston Conference on Biomedical Engineering Research. University of Houston, Houston, TX (February 13 - 14, 1997).

Wiederhold, M.L. "Supersonic snails." Slide presentation, Department of Otolaryngology-Head & Neck Surgery Alumni Day, University of Texas Health Science Center, San Antonio, TX (June 14, 1997).

Wiederhold, M.L. "Supersonic snails." Slide presentation, Department of Otolaryngology-Head & Neck Surgery Alumni Day, University of Texas Health Science Center, San Antonio, TX (June 14, 1997).

Wiederhold, M.L. "Development of gravity-sensing organs in microgravity." American Society for Gravitational and Space Biology. ASGSB Bull. (In Press).

Wiederhold, M.L., Gao, W., Harrison, J.L., and Hejl, R. "Development of gravity-sensing organs in altered gravity." ASGSB Bull. (In Press).

Wiederhold, M.L., Gilpatric, K.D., Hejl, R.J., Koike, H., and Nakamura, K. "Development of otoliths and endolymphatic otoconia in newt larvae reared in microgravity." Abstracts of the Nineteenth Midwinter Research Meeting of the Association for Research in Otolaryngology, 19:145 (1996).

Wiederhold, M.L., Hejl, R.H., Harrison, J.L., Gao, W., and Hortick, C.H. "Development of a molluscan gravity-sensing organ in microgravity." Fifteenth Annual Houston Conference on Biomedical Engineering Research, University of Houston, Houston, TX (February 13 - 14, 1997).

Wiederhold, M.L., Pedrozo, H.A., Harrison, J.L., Hejl, R., and Gao, W. "Development of gravity-sensing organs in altered gravity conditions: Opposite conclusions from an amphibian and a molluscan preparation." J. Grav. Physiol. (In Press).

Spaceflight Effects on Mammalian Development

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1998

Students Funded Under Research: 10

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303031

Flight Assignment: NIH-R1 (STS-66, November 1994) and NIH-R2 (STS-70, June 1995)

Responsible NASA Center: Ames Research Center

Task Description:

Dr. Alberts and colleagues will study the fetal and postnatal development of rats to verify the hypothesis that microgravity reduces stimulation of the developing fetal vestibular system and thereby alters early function. The studies will also emphasize the behavior and physiology known to contribute to successful pregnancy, labor, delivery, and onset of postnatal maternal care, especially lactation. The data expected can contribute to our understanding of basic vestibular function. This function plays an important role in numerous disorders of movement and coordination, rehabilitation processes after injury, and deterioration during aging. The data expected will also answer fundamental questions regarding mammalian development and pregnancy in space and the effects of weightlessness on birth and lactation.

Differential responses of perinatal rats to tilt and rotation were reported in the previous year. As a key to understanding these space flight effects on perinates, video recordings of pregnant dams during space flight and of ground-based controls were examined. The results of this analysis have led to a perspective on behaviorally-driven activation in early vestibular development that is perturbed by space flight from its normal balance of afferent stimulation. Whereas microgravity will "unload" the otoliths, these observations indicate that the rats' (dams and fetuses) labyrinthes are hyperstimulated, especially around the horizontal canal. Altered angular accelerations delivered to fetuses by the weightless dams' behavior could contribute to the perinates' responses.

The research conducted as part of NIH-R1 was primarily and foremost a series of investigations into basic biological processes. Many of the observations verified and extended our NIH-R1 findings. In both investigations, the basic biological processes under consideration relate to (a) the ability of the body of an adult female mammal to tolerate space flight challenges and maintain normal gestation, followed by vaginal delivery

during 1-G readaptation, and (b) the developmental status of a vestibular system that forms and begins to function in microgravity, i.e., in the absence of normal gravitational forces. Within each of these pursuits are embedded numerous more specific yet fundamental research issues.

Most basic biological studies bear on some practical considerations, and this is true of the R1 and R2 studies. In particular, these experiments provide a foundation for understanding how the vestibular and proprioceptive systems are established in mammals and how function is shaped and maintained throughout life. Naturally, the results of a single experiment only give a most preliminary glimpse, but the ramifications are immense. Vestibular function and dysfunction appear early in human life — beginning with births and then through the maintenance of posture and coordination. Fetuses with vestibular disorders are prone to breach birth. The elderly suffer many disastrous falls, many of which appear related to altered vestibular or proprioceptive function. Basic developmental studies utilizing gravitational manipulations give new insights into the forces that shape and maintain the vestibular system, and will undoubtedly contribute to the foundation of knowledge needed for effective treatments and therapies.

One practical aspect of this work applies to the utilization of the upcoming International Space Station. Most plans for the life sciences laboratories on the space station include reproductive and developmental studies. As we learn more about the female mammal's adaptive responses to space flight, we can better plan the facilities needed for developmental research on a long-duration facility such as the space station.

FY97 Publications, Presentations, and Other Accomplishments:

Alberts, J.R. and Ronca, A.E. "Behavior of pregnant rats in space: A key to interpreting spaceflight effects on perinates." (Abstract) *Dev. Psychobiol.*, 30(3), 240 (1997).

Alberts, J.R. and Ronca, A.E. "Rat pregnancy and parturition survive spaceflight challenge: New considerations of developmental consequences." *J. Grav. Physiol.* (In Press).

Alberts, J.R. and Ronca, A.E. "Rat pregnancy and parturition survive spaceflight challenge: New considerations of developmental consequences." International Gravitational Physiology Meeting, Copenhagen, Denmark (1997).

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S., and Galvani, C.D. "NIH.R2 Spaceflight effects on rat parturition and offspring vestibular function." (Abstract) *ASGSB Bull.*, 10(1), 29 (1996).

Burden, H., Zary, J., and Alberts, J. "Effects of space flight during pregnancy on rat uterine connexin-43 and connexin-26." *FASEB*, New Orleans, LA (1997).

Ronca, A.E. and Alberts, J.R. "Altered vestibular function in fetal and newborn rats gestated in space." *J. Grav. Physiol.* (In Press).

Ronca, A.E. and Alberts, J.R. "Vestibular function in perinatal rats gestated in microgravity." (Abstract) *Dev. Psychobiol.*, 30(3), 261 (1997).

Ronca, A.E. and Alberts, J.R. "Altered vestibular function in fetal and newborn rats gestated in space." International Gravitational Physiology Meeting, Copenhagen, Denmark (1997).

Phantom Torso

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$130,000

Joint Agency Participation: NASA/NASDA

Flight Information:

Experiment ID: 9307039

Flight Assignment: STS-91 (1998)

Responsible NASA Center: Johnson Space Center

Task Description:

A full phantom torso will be heavily instrumented to measure the radiation distribution at various organ levels. A relationship to the skin to organ level doses will be established. Model verification will then be made.

Investigations of the Effects of Microgravity on In Vitro Cartilage Calcification

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-04

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9304016

Flight Assignment: NIH-C2 (STS-66, November 1994); NIH-C7 (STS-77, May 1996)

Responsible NASA Center: Ames Research Center

Task Description:

The experiment will study the effects of space flight on cells from chicken embryos. Analyses of the crystals found in the bones of young chickens hatched from eggs flown in space have shown the presence of smaller hydroxyapatite, or cartilage, crystals and the absence of any change in mineral crystal properties compared with Earth-based controls.

In this experiment, a scientific model of naturally occurring cartilage (a cartilage matrix) will be used to simulate animal cartilage. The experiment focuses on mineral deposition or calcification of cartilage. This experiment will be used to compare the mineral formed in the microgravity of space with that formed on Earth. Cultures at two different stages of development will be fixed for analysis at five points during the flight, allowing evaluation of changes in cell proliferation, cell maturation, and mineralization of the cultures. Two additional cultures will be fixed after re-entry.

Results will provide direct insight into how calcification in cartilage and bone may be controlled in space. This knowledge is important prior to extended human stays on the space station and may also provide a better understanding of the events involved in normal bone development on Earth. Such understanding may eventually lead to the development of improved treatments for osteoporosis and other bone disorders.

The analyses of these cultures, completed in 1996, demonstrated that cultures flown in hypogravity failed to mature. Increased cell proliferation and failure of the chondrocytes to mature, noted in the flight cultures, occurred in marked distinction to the formation of chondrocyte nodules in ground controls. Mineral analyses

(FT-IR), x-ray diffraction, and Ca:DNA ratios showed that the flight cultures did not mineralize, while mineralization did occur in ground controls. The results of these studies are being combined for publication with those of Dr. Landis. The manuscript in progress will combine methodologies for studying chondrocytes and osteoblasts in hypogravity.

This study focuses on how cells regulate biomineralization. Results should provide insight into an extremely prevalent disease, osteoporosis. Although much of osteoporosis is associated with alterations in hormonal levels, disuse osteoporosis is not uncommon. Astronauts lose bone mass during short-term flight, and this "osteopenia" may not be different from the osteopenia that leads to increased fractures (osteoporosis). The research is designed to allow for understanding of the underlying mechanism of biologic calcification. When this is known, improved therapeutics may be developed; however, the flight research does not test therapeutic modalities. The benefit to the citizens in the US should be a clearer understanding of why bone loss occurs, the importance of weight bearing for prevention of osteoporosis, and in the future, the development of therapies to prevent fractures in an ever-growing elderly population. An important outcome of the study was the observation that loading is important for cell maturation, illustrating the significance of exercise in osteoporotic patients.

FY97 Publications, Presentations, and Other Accomplishments:

Boskey, A.L., Binderman I., Steiner D., and Doty S. "Effect of space flight on chondrocyte maturation." *Trans. Orthopaed Res. Soc.* (February, 1997).

Fitzgerald, J. and Hughes-Fulford, M. "Gravitational loading of a simulated launch alters mRNA expression in osteoblasts." *Exp. Cell Res.*, 228, 168-171 (1996).

Stability and Precision of Human Performance during a Spacelab Mission

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Troy J. Zarcone, Ph.D.; University of Kansas

Funding:

UPN/Project Identification: E910
Initial Funding Date: 1995
Students Funded Under Research: 3
FY 1997 Funding: \$105,000

Solicitation: 89-OSSA-13 (IML-2)
Expiration: 1997
Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 8913010
Flight Assignment: HP-01 (STS-89, January 1998)
Responsible NASA Center: Johnson Space Center
Flight Hardware Required: Powerbook 170 computers with keypads, floppy disks

Task Description:

The payload proposes to determine the stability and accuracy of cognitive and psychomotor performance across work shifts, to measure the subjective responses of crew members on emotion and disposition questionnaires across work shifts, and to determine the relationship between subjective responses and cognitive and psychomotor performance.

Progress over the past year has focused on delivery and testing of both the hardware and software for this project to the NASA Johnson Space Center in Houston. Training of the assigned flight crew on the performance battery was initiated and ongoing preflight data collection was undertaken to establish the stability criteria essential to the Baseline Data Collection (BDC) phase of the experiment. An automated data analysis procedure has been developed and the flight crew stability training performances are being evaluated in relationship to the pretest studies conducted in the laboratory during the previous reporting period (Life Sciences Task Book FY 96).

The research undertaken on this task will help provide a better understanding of the basic "fitness for duty" requirements that characterize job performance under a range of conditions both on Earth and in space. The research will also contribute to the development of an effective technology for assessing fitness for duty status with a valid and reliable testing instrument that can be administered under conditions that do not require special instruments, facilities, or long periods of time. The research will also increase our understanding of the relationship between self-report measures of subjective responses and objective measures of performance in the interest of developing a valid and reliable early warning system for timely intervention of countermeasures to performance decrements.

FY97 Publications, Presentations, and Other Accomplishments:

Brady, J.V., Hienz, R.D., Zarcone, T.J., and Kelly, T.H. "Assessing the stability and precision of performance during space flight." *Integrative Physiol. Behav. Sci.*, vol. 32, 79-180 (1997).

Kelly, T.H., Taylor, R.C., Heishman, S.J., and Crouch, D.J. "Performance measures of behavioral impairment in applied settings" in "Handbook on Drug Abuse." Edited by: Karch, S.B. CRC Press: Boca Raton (In Press).

*The Interaction of Microgravity and Ethylene on Soybean Growth and Metabolism***Principal Investigator:**

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 Dimitriy Klimchuk, Ph.D.; National Academy of Sciences of Ukraine
 Elizabeth Kordyum, Ph.D.; National Academy of Sciences of Ukraine

Funding:

UPN/Project Identification: not available
 Initial Funding Date: not applicable
 Students Funded Under Research: 8
 FY 1997 Funding: not applicable
 Joint Agency Participation: NSAU

Solicitation: not available
 Expiration: not applicable
 Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9600001
 Flight Assignment: CUE (STS-87, November 1997)
 Responsible NASA Center: Kennedy Space Center
 Flight Hardware Required: BRIC

Task Description:

Space flight has profound effects on plants including altered starch metabolism, increased ethylene production, and a change in biomass partitioning. However, the mechanism for and/or relationship between these changes are not clear. Therefore, as part of the Collaborative Ukrainian Experiment (CUE), we will test the hypothesis that ethylene concentrations will be enhanced in the space-grown soybean seedlings resulting in diminished root growth in these plants relative to the ground controls. Furthermore, we will test whether removal of the ethylene from the atmosphere around the plants will result in biomass partitioning similar between space flight and ground control. Additionally, we hypothesize that starch concentrations in the cotyledons will be reduced in the space-grown plants as a result of diminished AGPase activity. The level at which this enzyme is regulated will be investigated. We also hypothesize that the removal of ethylene from the atmosphere of the space-grown plants will result in starch concentrations similar to the ground controls.

Activities during the past year have been directed toward preparing for the scheduled flight of our experiment on STS-87 in late 1997. We have participated in a Science Verification Test and a Payload Verification Test, both of which are simulations of the entire mission. We have developed protocols for on orbit watering soybean seeds, gas sampling and storage, ethylene scrubbing, and specimen freezing. Additionally, we spent time training the Payload Specialists in background theory for the science and methodology for the successful

completion of the experiment. Finally, we completed several runs of an experiment measuring growth, gas composition, and carbohydrate metabolism in clinostat-grown soybean plants using the exact configuration of hardware as will be used for the flight. This allowed us to refine the techniques prior to the mission and will provide valuable ground-based data to which we can compare our flight results.

Results from these experiments, in addition to fostering a climate of international cooperation in space, will lead to a more complete understanding of the influence that space flight has on plant growth and metabolism. This is of critical importance as humans venture deeper (and longer) into space. Long-term missions will utilize plants as part of a life support system for the crew, and the influence that space flight has on plants must be understood. The information generated by these studies will not only result in a more thorough understanding of the influence of adverse conditions on plant growth and metabolism, but will also supply important information toward the development of a life support system using plants.

FY97 Publications, Presentations, and Other Accomplishments:

Brown, C.S. "The Collaborative Ukrainian Experiment: Scientific overview." 12th Man In Space Symposium, Washington, DC (June 13, 1997).

Brown, C.S., Sanwo, M.M., Hilaire, E., Guikema, J.A., Stryjewski, E.C., and Piastuch, W.C. "Starch metabolism and ethylene production in space-grown soybean seedlings." *ASGSB Bull.*, 10, 34 (1996).

Brown, C.S., Sanwo, M.M., Stryjewski, E.C., Peterson, B.V., Johnson, C.F., Piastuch, W.C., Hilaire, E., and Guikema, J.A. "Carbohydrate metabolism and growth in space-grown soybean." *Plant Physiol.*, 114, 91 (1997).

Brown, C.S., Sanwo, M.M., Stryjewski, E.C., Peterson, B.V., Johnson, C.F., Piastuch, W.C., Hilaire, E., and Guikema, J.A. "The interaction of microgravity and ethylene on soybean growth and metabolism." 12th Man In Space Symposium, Washington, DC (June 11, 1997).

Hilaire, E., Peterson, B.V., Guikema, J.A., and Brown, C.S. "Clinorotation affects morphology and ethylene production in soybean seedlings." *Plant Cell Physiol.*, vol. 37, no. 7, 929-934 (1996).

Johnson, K.M., Brown, C.S., and Piastuch, W.C. "Examination of the expression patterns of genes involved in carbon metabolism in soybean seedlings grown in space." *Plant Physiol.*, 114, 253 (1997).

Physiological Anatomical Rodent Experiment (PARE) 04: Flight Support

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 5-01161

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Joint Agency Participation: NIH

Flight Information:

Experiment ID: 9303008

Flight Assignment: NIH-R1 (STS-66, November 1994), NIH-R2 (STS-70, June 1995)

Responsible NASA Center: Ames Research Center

Task Description:

This experiment will use pregnant rats to determine the effect of space flight on ovarian antral follicles, corpora lutea, and pituitary content of hormones. These studies will provide insight on the role of gravity in hypophyseal-ovarian function and fecundity on Earth.

We have completed our morphometric analysis of all dam ovaries. Also, hormones in both the serum and the pituitary have been measured in all dams. Lastly, the analysis on the effects of space flight on postimplantation fecundity has been completed. We found no effect of space flight during the post-implantation period on any of the ovarian morphometric parameters evaluated, nor on vaginal birth or fecundity. The question of whether space flight initiated during the preimplantation period has any effect on embryonic survival needs to be addressed.

Female germ cells (oocytes) are contained in ovarian follicles. The fate of over 99% of ovarian follicles and their oocytes is a degenerative process known as atresia. The cause of atresia is not known, but this process must be rigorously controlled *in vivo* if female mammals are to retain their reproductive capacity. This study was designed to examine the effects of space flight on atresia of antral follicles. We learned that space flight during the post-implantation phases of pregnancy does not alter this important ovarian regulatory process. Also, space flight during this period of pregnancy does not alter the rate of fetal wastage.

FY97 Publications, Presentations, and Other Accomplishments:

Burden, H.W., Zary, J, Lawrence, I.E., Jonnalagadda, P., Davis, M., and Hodson, C.A. "Effects of space flight on ovarian-hypophyseal function in postpartum rats." *J. Reprod. & Fert.*, vol. 109, no. 2, 193-197 (1997).

Effects of Space Flight on Muscles and Nerves

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Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303019

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Animal Enclosure Module

Task Description:

This project is an extension of the original grant which was designed to study the role of gravity in the development of skeletal muscles in the thigh of rats. Animals for the original study flew on STS-66 as part of the NIH.R1 project. Data from the first experiment indicate that in fetuses exposed to microgravity from gestation day 9 through gestation day 20, muscles develop normally with respect to gross morphology. However, the normal developmental progression of RNA expression of skeletal muscle contractile isoforms is altered by development in space. This second series of experiments extends the findings of the original project through the addition of muscles of the lower leg, postflight data analysis at postnatal days 1, 3, 7, 10, 14, 21, and 35, and the use of a riboprobes and antibodies intended to elucidate mechanisms of those changes due to development in space. Thus, these data may provide insight into possible long term effects of development in microgravity on growth and maturation of skeletal muscle. Additional data were collected on adult hind limb muscles from the dams. Rats for this second experiment flew on STS-70 as part of the NIH.R2 project. Nulliparous pregnant female rats were used as the experimental animal. Launch occurred on gestation day 11. Landing of the shuttle occurred on gestation day 20. On the day of landing/recovery, four dams were anesthetized and underwent cesarean delivery. The remaining dams were allowed to deliver naturally.

Entire hindlimbs (G20-PN14) or individual soleus, extensor digitorum longus, medial gastrocnemius, and tibialis anterior muscles (PN21, PN35, dams) were fixed and frozen at KSC and shipped to the University for analysis. Preliminary data analysis for fiber cross-sectional area and fiber type in the medial gastrocnemius and soleus muscles of the dams revealed no change in mean fiber size of myosin heavy chain expression following 9 days of exposure to space flight. Hindlimbs and muscles from the fetuses and pups have been sectioned and are

being stained with antibodies for myogenin, MyoD, and myosin heavy chain isoforms for embryonic, neonatal, type I, type Iia, type Iib, and type Iix. *In situ* hybridizations will be performed on tissues using riboprobes for myogenin, MyoD, myosin heavy chain embryonic and neonatal isoforms from those time points that no longer express protein in order to differentiate between RNA transcription and translation as mechanisms for the cessation of protein expression through development.

The first experiment looking at the development of mammals during space flight has presented us with some fascinating results. Although subtle, the differences in gross morphology of skeletal muscles might have important implications into those factors that control the way muscles are shaped in development. This increased understanding of the basic biological processes may lead to better prenatal care on Earth. In addition, the difference in protein development may change the way we think about how all the proteins that make up skeletal muscle interact and are regulated. New data from this second experiment are intended to provide more insight into mechanisms of the control of development in addition to expanding the number and type of muscles involved in the study. This may lead to differences in treatment of muscle tissue following damage or disease.

Gravitational Effects on Embryogenesis in Poaceae

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-01

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$85,000

Flight Information:

Experiment ID: 9601066

Flight Assignment: BRIC-13 (STS-95, October 1998)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC

Task Description:

This research is based on the hypothesis that embryogenesis in higher plants is determined by the plane and direction of early cell divisions. These divisions determine axis polarity and ultimate embryo development. Gravity has a strong influence on these processes. Specific objectives are to study the role of hypergravity, hypogravity, and the actual space environment on embryo initiation and development. The strength of the investigations is based on the use of a unique and superior system, developed in our laboratory, for studying plant embryogenesis. Embryos initiate and develop directly from single mesophyll cells in cultured leaf segments of 'Embryogen-P' orchardgrass (*Dactylis glomerata L.*). Leaves are split along the midvein and segments from one half are used for a treatment while the corresponding sister or mirror segments from the other half serve as controls. The first part of the funding period will be spent on characterization of our system at the electron microscope (EM) level. These include studies on frequency of periclinal versus anticlinal divisions, polarity establishment, cell wall formation, cytoskeletal development, etc. Experiments during the first year will focus on explant orientation, clinorotation, and hypergravity disturbances of these events. These studies form an anchor for a repeat of our BRIC-02 flight experiment which we propose for the second year. Quantitative data will be collected on embryo yield and tissues will be fixed and processed for light microscope and EM investigations. This will be done for the ground-based research and the flight experiment. The third year will be used to analyze results from the flight experiment and relate them to those from our other gravitational studies. It is expected that ground-based experiments will also continue during this period.

Funding for the current project has been received for only two months (effective start date August 1, 1997). Experiments were initiated to study the cellular organization of two to eight cell stage proembryos in orchardgrass leaf segments.

Using the fluorescent dye, Hoechst 33258, we have begun investigations on both mitotic index and cell division planes in early proembryo cells. Initial observations confirm the hypothesis of a single cell origin of somatic embryos from mesophyll cells. These analyses support our earlier concept that the plane of division leading to

embryo formation is predominately periclinal, and that the maintenance of early embryo polarity is established during the first one to three cell divisions. Electron microscopic studies of microtubules in proembryo cells is providing information on their distribution during mitosis. Confocal microscopy of cells stained with acridine orange is being used for more detailed observations of mitosis.

A clinostat has been designed and is being constructed which will rotate leaf segments in a vertical position. This will provide a horizontal axis of rotation that is important for mimicking the effects of microgravity in ground-based experiments. The clinostat design allows for both slow (1 rpm) and fast (50-100) rotation.

The research utilizes a model system for studying the initiation and development of plant embryos. The species utilized is orchardgrass (*Dactylis glomerata L.*), which is in the same botanical family as the major cereals, corn, wheat, rice, etc. In this system, embryos initiate and develop directly from single somatic cells in leaf tissue. Results from a flight experiment, BRIC-02, showed a 70% reduction in embryogenesis from tissue plated 21 h prior to launch. Extensive histological analyses of tissues indicated that the direction and plane of the early cell divisions, perhaps even the first, are critical in embryo initiation and development. Specifically, if the first division is anticlinal (perpendicular to the surface) rather than periclinal (parallel to the surface), polarity may not be established, the axis may not be determined, and divisions may not continue to produce an embryo. It is anticipated that ground-based research involving clinorotation and hypergravity will supplement the microgravity (flight) experiments in answering fundamental questions on the importance of the direction and plane of cell divisions in plant embryogenesis, especially in the family *Poaceae* (grasses and cereals).

Results from our flight experiment have important implications for space travel. Embryos are an integral component of plant seeds. If embryos fail to initiate and develop, seeds are unlikely to form. This is of potential high significance in long-term space missions or on space stations where production of seeds may be needed, either for direct consumption (e.g., wheat or rice) or for growing another crop.

Gravity Effects on Seedling Morphogenesis

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10

Solicitation: 95-OLMSA-02

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$124,356

Flight Information:

Experiment ID: 9502023

Flight Assignment: BRIC-J (STS-95, October 1998)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC

Task Description:

In germinating cucumber seedlings, two morphogenetic processes appear to require gravity, namely, the formation of the hook in the apical region of the hypocotyl and the formation of a peculiar outgrowth, known as the "peg," at the transition zone between the hypocotyl and the root. The development of these organs involves a major change in the pattern of cell expansion. Independent work in our lab has identified a novel class of wall proteins, which we have dubbed "expansins," that catalyze cell enlargement by promoting the slippage and relaxation of the load-bearing network(s) in the wall. For this project, we propose:

- molecular cloning and sequencing of expansins associated with the development of the apical hook and the peg in germinating cucumber seedlings;
- characterization of expansin gene expression (at the mRNA and protein levels) during development of the apical hook and the peg;
- clinostat experiments to analyze how simulated weightlessness modifies the development of these organs and the expression of expansins; and
- a flight experiment, in which cucumber seedlings at various stages in development will undergo the transition to the micro-g environment of near-Earth orbit; these seedlings will be chemically fixed and frozen while in orbit and subsequently analyzed for alterations in morphology, cytology, and expansin gene expression in the hook and peg regions.

The first phase of this project involves cloning and characterizing the expression pattern of expansin genes expressed in the peg and root tissues of etiolated cucumber seedlings. Using an inverse PCR method, we have identified three unique expansin genes in these tissues. By Northern analysis, it appears that they have overlapping patterns of expression. Initial attempts at identifying expression patterns by *in situ* hybridization

were inconclusive because of high background problems. Current efforts are aimed at developing an *in situ* RT-PCR method for evaluating expression patterns for the three new expansin genes. Moreover, we are testing techniques for tissue freezing and fixing in preparation for the flight experiment.

The results of the ground-based experiments will yield greater insight into the action of expansins and their role in plant cell growth. This information will add to our understanding of the molecular mechanisms control plant cell growth, with the possibility of inventing new ways to genetically engineer plants for improved growth characteristics.

Development of Sensory Receptors in Skeletal Muscle

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 6

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303006

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Task Description:

In this study of rats that underwent part of their prenatal development in space, we are examining microscopically the formation of encapsulated sensory receptors — the two major types being muscle spindles and tendon organs — in one extensor and one flexor hindlimb skeletal muscle. We are determining the presence, number, and size of the muscle spindles when the rats are of different ages (i.e., fetal or gestational day 17, new born, and adult or postnatal day 100). An earlier part of our research tested for effects of space flight during late stages of gestation on the following postnatal events: weight gain, initiation of walking, eye opening, use of hind limbs during walking, and ability to reproduce. Comparisons are made with similar measures on ground-based control rats.

In FY97, we wrote a paper describing our findings for various physiological and behavioral features of the flight dams and their offspring relative to ground control rats. It will be published in *Integrative Physiological and Behavioral Science* (32/4:322-342. 1997). That part of the project is complete.

Also during FY97, we continued to add to the results of our microscopic analysis of encapsulated skeletal muscle receptors in the following two ways. We gathered data on muscle spindles in the extensor digitorum longus muscle (an ankle flexor) of 100 day old flight and control rats. Second, we are still in the process of examining the soleus (an ankle extensor) and the extensor digitorum longus muscles of flight and control rats that were fetuses and neonates. Some of those data are in the process of being analyzed. They will subsequently be prepared for submission to a refereed journal.

Encapsulated sensory receptors in skeletal muscles are important for the development and maintenance of normal somatic motor function. From an evolutionary perspective, typical muscle spindles and tendon organs seem to

have appeared in skeletal muscle when vertebrates became land dwellers. For example, encapsulated muscle receptors are not present in fish and most amphibians, but they do occur in trunk and limb muscles of all reptiles, birds, and mammals so far examined. This raises the question as to whether a markedly decreased gravitational field, as occurs in space, would alter the proximate causation conditions for development of encapsulated sensory receptors.

This study has shown that rats which undergo most of the latter part of their gestation — a time when the skeletal muscle receptors normally start to develop — in near-zero gravity, do begin motor behaviors (e.g., walking) on a normal developmental schedule after being born on Earth. The use of their hind limbs during walking also progresses normally with increasing postnatal age. Furthermore, muscle spindles and tendon organs do develop in hind limb muscles in these rats.

If these initial results continue to hold as our study progresses to completion, it would suggest the following fundamental conclusion. Gravity has little, if any, effect on the proximate cause mechanisms for the initial development of encapsulated sensory receptors in skeletal muscles of a rat. Said another way, at least in the short-term, a fetal mammal should be able to develop in space without risking adverse effects on formation of encapsulated receptors in skeletal muscle and the somatic motor functions they subserve when that animal is born and develops postnatally on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

DeSantis, M., Eldred, E., Helmick, C., Hines, J., and Wong, A. "Quantitative observations on the structure of selected proprioceptive components in adult rats that underwent about half of their fetal development in space." Presentation at the 12th Man in Space Symposium, Washington, DC (June, 1997).

Wong, A.M., and DeSantis, M. "Rat gestation during space flight: outcomes for dams and their offspring born after return to earth." *Integrative Physiol. & Behav. Sci.* (In Press).

The Effect of Spaceflight on Cartilage Cell Cycling and Differentiation

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Funding:

UPN/Project Identification: 106-50-01

Solicitation: 96-OLMSA-01

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$60,000

Flight Information:

Experiment ID: 9601378

Flight Assignment: STS-95 (October 1998)

Responsible NASA Center: Ames Research Center

Task Description:

Previous flight experience using isolated chick chondrocytes or rat osteoblasts has resulted in equivocal results. Part of the problem seems to center around the change in growth characteristics of these cells in the CellCo cartridges compared to growth in 35mm petri dishes. Nevertheless, even with these differences, the cells subjected to space flight do not seem to reach the same level of activity as the ground controls. This has been suggested by the reduced utilization of glucose and decreased lactate formation accumulated in the media, and the electron microscopic observation of reduced collagen formation following flight. There have been many studies of normal cells in culture, in which the cell proliferative phase is followed by cell differentiation, increased cell metabolism and reduced proliferation. This is what we would have expected from the cells undergoing space flight, but the differentiation step seems to be reduced from ground control activity.

There are several possible explanations for these space flight results: (1) There is an increase in cell proliferation but normal differentiation, with the proliferative phase involving the majority of cells in the population. (2) There is no change in proliferation, but cell differentiation is reduced due to some unexplained direct effect of space flight on a process in the differentiation pathway. (3) There is an increase in cell death resulting in reduced overall cell population. This reduction in cell pool size could result in a reduced metabolism of the flight cell population compared to controls.

We suggest studying these three possibilities using cartilage micromass cultures which are flown in the mid deck lockers using the Space Tissue Loss equipment developed by Walter Reed Army Institute of Research. Samples of cartilage are briefly fixed in 70% ethyl alcohol at 12 hours following launch, at 4 and 8 days of flight, and at recovery. The cartilage cells are collected and analyzed for DNA fluorescence with a flow cytometer, to determine the percentage of cells in S, G₀/G₁ and G₂/M phases. Cell differentiation will be analyzed by measurement of alkaline phosphatase fluorescence (flow cytometer), histochemistry (light microscopy), and morphology (electron microscopy). Cyclin expression as measured by flow cytometry and immunocytochemistry will be utilized to describe the chondrocyte cell cycle. Cyclins which can be used are

cyclin D2 and D3, cyclin E, and cyclin A. Proliferating cell nuclear antigen will also be used as an overall indicator of cell division. Cell death will be determined by nuclear fragmentation (flow cytometry) and apoptotic immunocytochemistry (light microscopy).

This project will utilize a combination of technologies to answer some questions unique to cell function here on Earth as well as during space flight. The application of flow cytometry and cell labeling with cell cycle indicator proteins will open up new methods of study of cell cycling in experimental and pathological conditions. Certainly this allows us to determine how cell populations are behaving (i.e., undergoing cell division, experiencing cell death and/or apoptosis, cell arrest in one phase of the cell cycle, etc). This information is a prelude to understanding whether cells are differentiating towards maturity or remaining in a stem cell or undifferentiated state. This state determines cell and tissue behavior and ability to respond to signals, such a cytokine or hormonal stimulation. In the final analysis, "normal" or "pathological" responses are dictated by the cell's ability to cycle normally and to differentiate fully. It will be of great interest to determine whether space flight has any impact on this cell cycling. A flight effect could explain many results from plant and animal experiments which have previously flown. An effect could also lead us to use this new information for the study of aging, osteoporosis, arthritis and other connective tissue diseases to determine whether similar findings are present on Earth.

Although this project was chosen in December 1996 for definition, no NASA funding has been made available. Therefore there is no progress to report at this time.

Genetically Engineered Plant Biomonitors in Microgravity

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-05

Initial Funding Date: 1994

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9305020

Flight Assignment: PGIM-01 (STS-93 [Target])

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: PGF

Task Description:

The purpose of this project is to develop state-of-the-art transgenic plant technology to answer important questions regarding plant biology in microgravity environments. We are developing a series of transgenic plants that will act as biological monitors of the conditions perceived by plants in microgravity. This is being accomplished by genetically engineering plants such that they contain specific environmental response genes designed to register and report the plant's perception of the environment.

The genetically engineered biomonitor plants are called TAGES, for Transgenic *Arabidopsis* Gene Expression System. They contain alcohol dehydrogenase promoter derivatives driving the GUS reporter gene.

From past flights of the TAGES biomonitors plants on the KC-135 research aircraft out of Johnson Space Center, we were aware that there were qualitative changes in reporter gene expression in plants experiencing parabolas exhibited marked expression of the reporter gene, indicating the onset of aberrations similar to those reported from shuttle missions. In addition, a series of centrifuge experiments were conducted in order to rule out the periods of 2-G as possible causes of the activation of the stress response. TAGES plants were subjected to a 1- to 2-G centrifugation series which mimicked the hyper-G portion of the parabolic flights. TAGES plants were subjected to 80 truncated "parabolas," each truncated parabola going from 1-G to 2-G over a time frame similar to that of the KC-135 recovering from a dive, while resting at 1-G for the time that would be microgravity on the KC-135. The centrifuged plants demonstrated no visual qualitative differences from the plants maintained as controls.

Recent full analysis of the quantitative levels of reporter gene activity in modified gravities extends these data. All of the centrifuge plants, both experimentals and controls, had quantitative reporter activities similar to the

ground controls from the KC-135 flights, whereas the KC-135 flight plants exhibited reporter gene activities that were 4- to 5-fold greater than ground or centrifuge controls. These results offer stronger quantitative support for proof of the concept that reporter genes can be used to monitor the wide-range of alterations that space-flight experiences impart on plants. In addition, these results confirm by quantitative measures that at least some of the early stages of the alterations that apparently occur in space flight can be examined in atmospheric parabolic flights and centrifugation series. The ultimate goal is to use this information to help design growing systems and media that reduce or eliminate stress responses.

Like all living organisms, plants constantly monitor their environment and make adjustments to their physiology as environmental needs dictate. Changes in environmental conditions almost uniformly lead to changes in gene regulation in plants, and these regulatory adjustments provide the altered molecular condition within the plant cell that allows the plant to survive and even grow in the new environmental situation. For example, plants exposed to microgravity conditions have shown ultrastructural characteristics that are similar to terrestrial plants exposed to hypoxia, but it is unknown whether the plant is actually responding to reduced oxygen potential or some secondary effect of microgravity. This research is dedicated toward the engineering of plants that are capable of reporting their perception of potentially adverse environmental situations to the investigator. Data from these plants as well as the entire experimental approach might also be used to examine the cause of certain plant growth anomalies that have resulted from exposure to adverse environmental conditions on Earth. Effective evaluation and dissection of plant genes, together with the development of tailored reporter gene systems, can provide the scientific community with plants capable of monitoring the growth conditions actually perceived by plants in adverse environments on Earth or in space.

Protein Turnover During Space Flight

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Funding:

UPN/Project Identification: 106-50-10
Initial Funding Date: 1997
Students Funded Under Research: 0
FY 1997 Funding: \$75,000
Joint Agency Participation: UTMB

Solicitation: 95-OLMSA-01
Expiration: 2000
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9501048
Flight Assignment: PTO (STS-95, October 1998)
Responsible NASA Center: Johnson Space Center

Task Description:

Increased protein turnover has been documented during SLS-1 (Stein et al., 1994), ostensibly due to a stress response. However, the dynamic relationship between the endocrine response and whole-body protein turnover has not been assessed in flight. Furthermore, ground-based studies are not appropriate models for investigating protein turnover in microgravity (Ferrando et al., 1995). This study will test the hypothesis that microgravity increases whole body protein turnover due to alterations in cortisol and insulin concentrations or action. By establishing this relationship in microgravity, we will be able to construct appropriate ground-based models that allow the testing of operational countermeasures. Whole body protein turnover will be determined by the appearance of ¹⁵N enrichment of urea and ammonia in blood and urine samples after consumption of ¹⁵N alanine. Muscle protein breakdown will be assessed by intravenous administration of 3-methyl (2H3)-histidine. Blood cortisol and insulin concentrations will be determined to better understand neuroendocrine indices of protein metabolism during space flight. Body composition will be determined before and after flight; dietary intake will be assessed before and after flight through the use of weighed food records, and during flight with a bar code reader. We propose to determine the effect of continued space flight (3 to 16 days) on whole body protein turnover, and will determine the rate of recovery after return to Earth as well.

This project has been tentatively assigned a Shuttle flight for the fall of 1998. We have continued work on the refinement and analysis of 3-methyl-histidine in blood. Work has focused on the adaptation of existing kinetic models of 3-methyl-histidine in the blood such that blood sample amounts and time points can be reduced for flight purposes. We have endeavored to purchase a new GCMS system which will greatly increase the analytical sensitivity and enhance our detection capabilities. This equipment should be functional within 6 months. All other methods are in place and have been used extensively in our laboratory.

These studies will provide crucial information for the establishment of a viable ground-based model to study the perturbations of protein metabolism during space flight. In turn, this will provide the capability of testing potential countermeasures and determining nutritional requirements for future extended duration missions. Most importantly, studying the effects of stress on an otherwise healthy population will enhance our understanding and treatment of the clinically stressed patient on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Ferrando, A.A. and Wolfe, R.R. "Effects of bed rest with or without stress" in "Physiology, Stress, and Malnutrition." Edited by: Kinney, J. and Tucker, H. Lippencott-Raven: New York, pp 413-430 (1997).

Ferrando, A.A., Tipton, K.D., Bamman, M.M., and Wolfe, R.R. "Resistance exercise maintains skeletal muscle protein synthesis during bed rest." *J. Appl. Physiol.*, vol. 82, no. 3, 807-810 (1997).

Ferrando, A.A. "Muscle protein synthesis following simulated weightlessness in humans." American College of Sports Medicine, Denver, CO (May, 1997).

Ferrando, A.A. "Net protein synthesis and amino acid uptake with testosterone injection." *Experimental Biology*, New Orleans, LA (April, 1997).

*Evaluation of Thermoregulation During Short-Duration Space Flight***Principal Investigator:**

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 John Greenleaf; NASA Ames Research Center
 Richard Gonzalez; U.S. Army Res. Instit. Environ. Med.
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Funding:

UPN/Project Identification: 106-50-10	Solicitation: 95-OLMSA-01
Initial Funding Date: 1996	Expiration: 1999
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$50,000	

Flight Information:

Experiment ID: 9400322
 Flight Assignment: ETSF (STS-95, October 1998 [Target])
 Responsible NASA Center: Johnson Space Center

Task Description:

The purpose of this study is to evaluate thermoregulatory responses of shuttle crew members before, during, and immediately after space flight. This flight investigation is currently in the queue awaiting a flight opportunity to be manifested as a small payloads study.

In 1996, we began ground-based definition studies to further validate the use of the telemetry pill system during bedrest and to evaluate changes in thermoregulation during microgravity simulation. This study will determine whether greater heat storage during exercise after simulated space flight (bedrest) is due to impaired skin vasodilation or impaired sweating. Before and immediately after 13 days of bedrest, 8 subjects will perform a submaximal, supine exercise test (20 min each at 40% and 65% pre-bedrest VO_{2max}) with measurements of core temperature (esophageal and gut temperatures), skin temperatures (thermistors), skin perfusion (laser Doppler flowmeter and forearm strain gauge), and local chest sweating (dew point hygrometry). Changes in thermal responses will be related to changes in blood volume during the bedrest, as measured via isotope dilution (125I labeled human serum albumin and 51Cr labeled sodium chromate).

This study is undergoing definition for possible manifesting on the upcoming STS-95 Shuttle mission.

The results of this study will have direct benefits to assessing the potential for heat-induced injuries for crew members during flight and recovery from flight. Significant Earth-based benefits may be realized by application of the non-invasive methodology used in this study to measure body temperatures. The miniaturized telemetry

system used in this study is being upgraded from currently available commercial systems. The flight-qualified system will be small and easier to use and much less susceptible to data dropouts due to the poor design of the original antenna system and to susceptibility of the receiver to electromagnetic interference. Small, portable "heat strain" indicators have direct application to conditions in which humans must work in extreme environments or while wearing heat-impermeable clothing: firefighters, soldiers in chemical warfare garments, workers in nuclear/chemical protection garments, etc.

Effects of Weightlessness on Vestibular Development in Rat Pups

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Funding:

UPN/Project Identification: 106-50-06

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303003

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Task Description:

The lack of gravity is known to alter vestibular responses in developing and adult vertebrates. One cause of these altered responses may be changes in the connections between the vestibular receptor and the brain. Therefore, we propose to investigate the effects of gravity on the formations of connections between the gravity receptors of the ear and the brain in rat pups raised in space beginning at an age before these connections are made until near the time of birth, when they are to some extent functional. This investigation will make use of a novel technique, the diffusion of a lipophilic dye, DiI, in fixed tissue. This technique can be used to analyze the connections in specimens fixed immediately after landing of the space shuttle, thus minimizing changes due to the Earth's gravity. The evaluation of the data will enable us to detect gross deviations from normal patterns as well as detailed quantitative deviations.

Thus far we have studied about two thirds of the flown embryos. Our data clearly indicate several effects of microgravity on neuronal development, two of which were already published as abstracts. The third major effect needs further confirmation using remaining flight and control material.

Questions answered are: (1) The absence of gravity affects the rate of maturation of the gravistatic projections to the brain. The saccular and vestibular connections appear less mature in the flight animals than in the synchronous controls. (2) In control animals, some ganglion cells in the geniculate ganglion project to the inner ear but many more do so in the flight animals. (3) In both normal and flight animals efferent fibers have branches to both the saccule and the cochlea. The occurrence of multiple branches has not previously been reported in normal rats, and was an unexpected finding.

As a result of these findings we are now seeking answers for two new questions:

A) Does the projection of the non-gravistatic vestibular receptors of flight animals mature even faster than that

of control animals? In other words, is there a reciprocal effect of microgravity on the gravistatic versus non-gravistatic projections of the vestibular system?

B) What is the degree of maturation of synaptic contacts between vestibular fibers and second order neurons?

Thus far we have found that there are fewer mature synaptic contacts between the saccular fibers and the vestibular nuclei in microgravity exposed animals. In contrast, the semicircular canal afferents show no qualitative differences between flight and control animals. We have also calculated the total numbers of synapses in vestibular nucleus areas in flight and control animals and our data show that the overall numbers of synapses is highly variable between different embryos and even between non-consecutive sections taken from the same embryo. No statistically significant changes in numbers of synapses have been found in the vestibular nuclei of flight and control animals. In combination with the apparent changes in fiber pathways, this would indicate that under microgravity conditions, semicircular fibers take over postsynaptic sites normally occupied by gravity responsive fibers.

The results of our research should allow us to obtain answers to the more general question, namely, is there a critical period during which the gravistatic and non-gravistatic components of the vestibular system compete for the targets in the vestibular nuclei as demonstrated in other maturing sensory systems.

Our data have opened an exciting new avenue of research into the anatomical basis of microgravity-related orientation deficits. Our study examined fetuses exposed to microgravity during a period when the vestibular system is just beginning to function. Our results show that fetuses exposed to microgravity have less mature gravity-sensitive projections compared to normal fetuses. Similarly, behavioral studies show that their littermates have similar behavioral deficits, and furthermore regained partial or complete responsiveness to gravity over time. We predict that these flight-induced anatomical alterations will be age related, and will be more pronounced and possibly permanent in animals exposed to microgravity soon after birth. Further anatomical and behavioral experiments are necessary to identify a possible critical period where gravity may be essential for the development of normal vestibular connections in neonatal rats.

If our continuing analyses support this hypothesis, we hope to identify a developmental period during which some gravistatic stimulation must be provided to ensure the development of proper connections between the gravistatic receptors in the ear and the vestibular nuclei in the brain.

FY97 Publications, Presentations, and Other Accomplishments:

Bruce, L.L., Christensen, M.A., and Fritsch, B. "Electron microscopic differentiation of directly and transneuronally transported DiI and applications for studies of synaptogenesis." *J. Neurosci. Meth.*, 73, 107-112 (1997).

Bruce, L.L., Kingsley, J., Nichols, D.H., and Fritsch, B. "The development of vestibulocochlear efferents and cochlear afferents in mice." *Int. J. Dev. Neurosci.*, 15, 671-692 (1997).

Fritsch, B. "On the role played by ontogenetic remodeling and functional transformation in the evolution of terrestrial hearing." *Brain, Behav. Evol.*, 50, 38-49 (1997).

Fritsch, B., Farinas, I., and Reichardt, L.F. "Lack of NT-3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region specific fashion." *J. Neurosci.*, 17, 6213-6225 (1997).

Fritsch, B., Silos-Santiago, I., Bianchi, L., and Farinas, I. "Neurotrophins, neurotrophin receptors and the maintenance of the afferent inner ear innervation." *Semin. Cell & Dev. Biol.*, 8, 277-284 (1997).

Fritsch, B., Silos-Santiago, I., Bianchi, L., and Farinas, I. "The role of neurotrophic factors in regulating inner ear innervation." *TINS*, 20, 159-165 (1997).

Effect of Spaceflight on the Development of the Circadian Timing System

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Funding:

UPN/Project Identification: 106-50-10 Solicitation: 93-OLMSA-03
Initial Funding Date: 1994 Expiration: 1997
Students Funded Under Research: 9 Post-Doctoral Associates: 0
FY 1997 Funding: \$0
Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303024
Flight Assignment: NIH-R2 (STS-70, June 1995)
Responsible NASA Center: Ames Research Center

Task Description:

Animals have evolved and developed within the constant gravitational environment of the Earth and the dynamic circadian changes in the environment associated with the 24-hour day. The circadian timing system (CTS) is an important temporal organizer controlling both the physiology and behavior of organisms. For example, conditions such as jet lag, shift work, and some sleep and mental disorders are frequently associated with dysfunction of the CTS. Our previous studies have shown that exposure of both mature and developing animals to hyperdynamic fields via centrifugation significantly affects the CTS. In addition, mature animals exposed to the microgravity environment of space flight exhibit altered CTS function. Although previous studies have demonstrated that exposure to space flight during the prenatal period can significantly delay a few general parameters of development, it is not known whether prenatal exposure to space flight will significantly alter maturation of the central nervous system, physiology, and behavior. This research will begin to examine the anatomy and physiology of the CTS of animals exposed to space flight during the prenatal period. These studies will focus on four areas: (1) the laminar development of the retina which provides visual pathway to the CTS; (2) the development of soma size and oxidative metabolism of neurons within the suprachiasmatic nucleus (SCN), the circadian pacemaker of the CTS; (3) the development of photic responsiveness of the SCN; and (4) the development of temperature and activity rhythms to examine the onset and maturation of circadian function. The retina and CTS provide excellent models for central nervous system development due to their well-characterized neural development and regulatory function in physiology and behavior.

During fiscal year 1997, we have continued to process and analyze the large amount of tissue and data collected for this program. Two preliminary reports of our observations were made during this period. We have been

processing the retinal and brain tissue. We have been analyzing this tissue in order to determine the effect of prenatal exposure to space flight on the development of the CTS.

This research examines the anatomy and physiology of the CTS of animals exposed to space flight. These studies may be useful for understanding the effect of an environmental stressor on prenatal development. These results may be usefully applied to many conditions where stress during pregnancy can affect the developing fetus.

FY97 Publications, Presentations, and Other Accomplishments:

Fuller, C.A. "Neural mechanisms of circadian rhythms after spaceflight." Invited presentation at American Psychological Society, San Francisco, CA.

Fuller, C.A., Hoban-Higgins, T.M., Murakami, D.M., and Tang, I-H. "Effect of pre-natal exposure to microgravity on circadian rhythms in rats during spaceflight." *ASGSB Bull.* 10(1):32 (1996).

Murakami, D.M., Hoban-Higgins, T.M., Tang, I.H., and Fuller, C.A. "The effect of flight NIH.R2 on retinohypothalamic tract development." *ASGSB Bull.* 10(1):32 (1996).

Murakami, D.M., Tang, I-H., Hoban-Higgins, T.M., and Fuller, C.A. "The effects of spaceflight NIH.R2 on the development of the circadian timing system." Presented at the 18th Ann. Int. Soc. for Grav. Physiol., Copenhagen, Denmark.

Effects of Altered Gravity on the Photosynthetic Apparatus

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1994
Students Funded Under Research: 3
FY 1997 Funding: \$90,703
Joint Agency Participation: NSAU

Solicitation: not available
Expiration: 1998
Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9600002
Flight Assignment: CUE (STS-87, November 1997)
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: PGF

Task Description:

Photosynthesis is the single most important contributor to food and fiber production, and is susceptible to a variety of stresses which can limit plant yields. This process must play a crucial role in a closed renewable life support systems during long space flight missions, for food, atmosphere, and water regeneration, and it will be critical to understand the effects of the space environment on the dynamics of such a complex process. Space missions in the past have provided tantalizing, albeit often anecdotal, glimpses of the effects of microgravity on cellular morphology and cell division and elongation, and how these effects may impact photosynthetic physiology. Differences in cell size, shape, division and elongation rates have been noted, as well as effects on respiration rate and on differentiation and cellular development, such as on plastid distribution and structure.

At present there are only few data concerning structure and functioning of chloroplasts in microgravity, and they are often contradictory. Space-grown tissues often show a decrease in thylakoid membrane stacking, and an increased number of plastoglobuli, indicative of thylakoid membrane turnover. Reports of the pigment content of space-grown tissues are contradictory, with some groups demonstrating an increased chlorophyll content in space-grown pea, while others suggest the opposite. Both chlorophyll and carotenoid levels of maize were reduced after growth for 19 d on Mir, and levels of pigment were reduced 35 to 50% in *Chlorella*. Kordyum and colleagues have suggested that changes in membrane structure may in part account for altered plant cell morphology during space flight. Alterations in membrane fluidity, mediated by changes in fatty acid composition or in the level of stress-induced lipid peroxidation, could have a profound effect on resource partitioning across membranes and mechanisms of chemiosmotic energy conservation. In recent work, Tripathy and coworkers monitored several aspects of photosynthetic function from wheat grown in microgravity. They found decreases in light-driven electron transport capacity of 25% at saturating light intensities.

Until space-based methods are available for the physiological assessment of plant processes such as photosynthesis, we are limited to an examination of materials which have been grown in space and either fixed/frozen or returned to Earth for rapid examination following landing. A "tissue sharing" experiment will examine the leaves of *Brassica rapa* for their photosynthetic characteristics after growth in the shuttle-based Plant Growth Facility. Preliminary experiments explored the design and configuration of rooting substratum, nutrient demands by the plants, temperature optima, etc., to assure optimal plant growth. Preliminary experiments also optimized procedures (electrophoresis, Western blot analysis, fluorescence kinetic assessments, etc.) which were developed on other species for *Brassica*. Plants will be grown aboard shuttle, a subset will be harvested and frozen or fixed, and a subset will be returned to Earth for physiological, cellular architecture, and biochemical studies.

A. Assessment of hardware and plant growth conditions

The Science Verification Test of October 1996, revealed that more work was necessary to assure that optimal growth of *Brassica rapa* would be obtained within the duration of the mission. In essence, seeds are planted in 'envelopes' which are inserted into grooves within foam blocks. Seeds are fixed into the correct orientation, and the payload specialist initiates growth and development by addition of a nutrient solution. Several nutrient solution mixtures were tested between the SVT and PVT, and harvest data was used to select for optimal growth.

B. Payload Verification Test

In April-May, 1997, a Payload Verification Test (PVT) was performed at the KSC. Modifications in plant nutrition, along with mission considerations such as total mass constraints, etc., allowed KSC engineering team to devise a plant watering strategy which employed water produced aboard shuttle to be passed through a filtering system to remove iodine from the shuttle check valve, mixed with a concentrated nutrient stock solution and after complete mixing, to be applied to the seed packets in the PGF. This strategy was successful in the PVT trials, and a recommendation that the payload initiation be delayed one day (to mission day 2) was accepted.

PVT activities also involved the testing of various fluorescence induction monitoring units, and how their use should be standardized and optimized for consistency. After the PVT, considerable ground-based research explored the types of information which fluorescence measurements can provide, and what quantitative variables like leaf size, etc, can be used in interpreting these measurements.

C. Additional ground-based research explored the use of *Brassica* in biochemical measurements of photosynthesis, such as in oxygen electrode studies and in Western blot assessments of photosynthetically active proteins.

Photosynthesis is the single biological process for the energetic capture of sunlight and its storage in chemical form. As such, it is critical for food and fiber production, and the understanding of this basic process is necessary for the development of strategies for sustainable and ecologically sound systems of agricultural production. Considerations of photosynthetic efficiency are especially important, since this process utilizes carbon dioxide as a substrate. Global climate changes are predicted as a result of fossil fuel-induced increases in atmospheric carbon dioxide, and this is also a characteristic of the atmosphere aboard the shuttle.

FY97 Publications, Presentations, and Other Accomplishments:

Brown, C.S., Sanwo, M.M., Hilaire, E., Guikema, J.A., Stryjewski, E.C., and Piastuch, W.C. "Starch metabolism and ethylene production in space-grown soybean seedlings." American Society for Gravitational and Space Biology Annual Meeting, Charlotte, NC (October, 1996).

Brown, C.S., Sanwo, M.M., Piastuch, W.C., Peterson, B.V., Stryjewski, E.C., Hilaire, E.M., and Guikema, J.A. "The interaction of microgravity and ethylene on soybean growth." 12th Man in Space Symposium, Washington, DC (June, 1997).

Brown, C.S., Sanwo, M.M., Stryjewski, E.C., Peterson, B.V., Piastuch, W.C., Johnson, C.F., Hilaire, E., and Guikema, J.A. "Carbohydrate metabolism and growth in space-grown soybean." Joint Annual Meetings of the American Society of Plant Physiologists and the Canadian Society of Plant Physiologists (August, 1997).

Guikema, J.A. "A Kansas nurseryman in space? Why not?" Kansas Nurseryman's Association, Manhattan, KS (August, 1997).

Guikema, J.A. "The Cooperative US/Ukrainian Experiment: The photosynthesis perspective." Emporia State University Division of Biological Sciences (September, 1997).

Guikema, J.A., Kordyum, E.L., Hilaire, E., and Leach, J.E. [invited symposium presentation] "History of the Cooperative U.S./Ukrainian Experiment (CUE)." 12th Man in Space Symposium, Washington, DC (June, 1997).

Hilaire, E., Paulsen, A.Q., Brown, C.S., and Guikema, J.A. "Plastid distribution in columella cells of a starchless *Arabidopsis* mutant grown in microgravity." *Plant Cell Physiol.*, 38, 490-494 (1997).

Hilaire, E., Peterson, B.V., Guikema, J.A., and Brown, C.S. "Clinorotation affects morphology and ethylene production in soybean seedlings." *Plant Cell Physiol.*, 37, 929-934 (1996).

Juergensmeyer, M.A., Odom, W.R., and Guikema, J.A. "The plastid-cytoskeleton interface: An immunological approach." American Society for Gravitational and Space Biology Annual Meeting, Charlotte, NC (October, 1996).

Leach, J.E., Chittoor, J., Hilaire, E., Jianfa, B., Lloyd, L., McGee, J.D., Panciano, G., Vera Cruz, C., Zhu, W., Guikema, J., and White, F. "Defense responses in rice to bacterial pathogens." Annual meetings of the Rockefeller Foundation. Singapore, Malaysia (August, 1997).

Wu, Y., Hilaire, E., Li, Q., Guikema, J.A., Brown, C.S., Piastuch, W.C., and Li, Y. "Seedlings grown at micro-gravity environment exhibit enhanced expression of auxin-regulated GH3 gene." Joint Annual Meetings of the American Society of Plant Physiologists and the Canadian Society of Plant Physiologists (August, 1997).

Bioavailability and Performance Effects of Promethazine During Space Flight

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Funding:

UPN/Project Identification: 106-50-01	Solicitation: 96-OLMSA-01
Initial Funding Date: 1997	Expiration: 1999
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$45,000	

Flight Information:

Experiment ID: 9601389
Flight Assignment: BPEP (TBD)
Responsible NASA Center: Johnson Space Center

Task Description:

Promethazine (PMZ) is the antinotion sickness drug of choice in the U.S. Space Shuttle program; however, virtually nothing is known about the bioavailability and performance effects of this drug in the microgravity environment. PMZ has detrimental side effects on human performance on Earth that could affect Shuttle operations. Although the side effects of PMZ given intramuscularly (IM) in space are reportedly less severe than those experienced on Earth, no objective measurements have been made to assess the potential effects of PMZ on performance in space. Moreover, symptoms of space motion sickness (SMS) have not been studied with regard to performance in space.

The proposed research will be conducted using the Portable In-flight Landing Operations Trainer (PILOT), a Shuttle landing simulator developed for in-flight astronaut training. Our objective is to establish the bioavailability and performance effects of intramuscular PMZ during flight and to examine the impact of SMS on crew performance. Results of this research should provide the necessary information to develop a safe and effective regimen of intramuscular PMZ for the treatment of SMS and to establish the applicability of PILOT simulations to assess potential performance decrements during an actual mission.

The research will be conducted in two phases: ground-based and in-flight. In the ground-based portion of the study, we will examine the applicability of using the performance impairment results from the PILOT to evaluate the pharmacodynamics of PMZ. Eight commercial pilots will serve as test subjects for this phase. Each will receive a 50-mg intramuscular dose of PMZ. Saliva and urine samples will be collected at regular intervals while using the PILOT. The effect of drug concentration on PILOT test scores will be examined to evaluate the pharmacodynamics of PMZ. The final in-flight protocol will be developed from the results of the ground-based study.

Data collection for the first ground-based study was completed. Nine subjects participated in this investigation, 6 commercial aircraft pilots and 3 Shuttle simulator trainers from Johnson Space Center. Subjects participated in four training sessions to achieve stable performance on Shuttle landings using the PILOT. The study was a double-blind cross-over design where each subject received Promethazine 50 mg IM in one session and Placebo IM in the other sessions; the drug/placebo order was counterbalanced. Data analysis of PILOT performance parameters and of saliva and urine for pharmacokinetic parameters is currently in progress, but not far enough along to report any statistical results at this time. However, "quick look" inspection of composite performance scores from the PILOT indicates the largest performance decrement occurs at 2-4 hrs following Promethazine administration. In addition, there appears to be much greater variability in performance scores (six landings at each of a number of specified times post-drug) following Promethazine than following placebo.

This preliminary investigation examined the effects of promethazine on PILOT performance when all approach and landing conditions were nominal. A new question that has arisen is: How is performance affected when conditions are off-nominal? A follow-up study to address this question is under consideration for FY98.

Examination of pharmacokinetics during space flight may yield new insights into pharmacokinetic processes on Earth. In addition, this research may serve a catalyst to identify new, more effective drugs for treating all forms of motion sickness which do not have the central nervous system (CNS) side effects of drugs currently used to treat this common malady. Motion sickness medications without CNS depressant effects could prove very beneficial not only to the general population, but to aircraft and shipboard crews that require treatment for motion sickness that does not interfere with the performance of critical tasks.

Application of Physical and Biological Techniques in the Study of the Gravisensing and Response System of Plants

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10
 Initial Funding Date: 1996
 Students Funded Under Research: 1
 FY 1997 Funding: \$ 108,418

Solicitation: 95-OLMSA-01
 Expiration: 1999
 Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9501335
 Flight Assignment: MFA-1 (TBD)
 Responsible NASA Center: Kennedy Space Center
 Flight Hardware Required: MFA

Task Description:

This continuing program combines ground-based research with the Small Payload Shuttle program. The core of the proposal is the study of the role of statoliths in the process of gravity sensing and response system in roots and shoots of higher plants by applying directional stimuli using high gradient magnetic fields (HGMF). The **gravisensing** system, specifically the role of amyloplasts, will be studied by applying directional stimuli using HGMF which enable the displacement of amyloplasts. As has been demonstrated earlier, HGMF exert a directional, repulsive force on diamagnetic substances such as starch in amyloplasts. We have shown that *in vitro* and *in vivo* amyloplast move along the gradient of the magnetic field. Microgravity experiments will establish threshold levels of the magnetic field strength, and thus force, required for root curvature. New experiments will test whether the force exerted by amyloplasts or their position inside sensory cells controls the direction of growth. The **graviresponse** system (i.e., changes in differential elongation) will be studied by investigating the behavior of the microtubule and actin cytoskeleton in ground and microgravity conditions. We will use μg -grown, fixed and HGMF-manipulated roots to establish whether the organization of the cytoskeleton is affected by μg . Typically, elongation of cells is characterized by deposition of cellulose microfibrils that are usually aligned perpendicular to the direction of growth. This is thought to enable differential elongation of cells and thus graviresponding organs. The research program will provide insight in the organization and operation of the gravi- sensing and -response system of plants.

The program progressed as a two tiered approach, the design of flight hardware and continuing ground-based research on the effect of HGMF.

The development of hardware resulted in a design of chambers that house two rows of five Neodymium/Iron/Boron magnets each, connected by two external yokes. The gaps between the magnets contain ferromagnetic wedges for the generation of the HGMF and seed holders. The holders accommodate four spaces for two seeds each with a fluid circulation system that provides water for germination and fixative for

termination of the space experiments. Live growth measurements will be done by an automatic, track-mounted video imaging system.

Research on the effect of microtubular and actin inhibitors showed that stabilization or depolymerization of these two components of the cytoskeleton has little effect on the graviresponse system. Taxol (MT stabilizer) treatment resulted in the appearance of abnormal spindle structures in dividing cells and swelling of the elongation zone and promoted the earlier than usual formation of oblique MTs. Despite unusual cell shape MTs maintained a highly organized transverse pattern. The MT depolymerizer oryzalin caused complete dissolution of MTs. Despite their effects on the MTs, neither taxol or oryzalin inhibited the graviresponse of roots. The actin microfilament (MF) organization is also correlated with differential elongation of primary maize roots. Vertically or horizontally oriented, rhodamine phalloidin stained roots showed that the organization of MFs of graviresponding roots was similar to vertical roots. Application of cytochalasin B or D; resulted in extensive disruption of MFs in the cortex and epidermis but only partially affected MFs in the stele. Despite the depolymerization of MFs, gravicurvature was not affected. In contrast, the auxin transport inhibitor *N*-1 naphthylphthalamic acid suppressed root curvature but had no observable effect on the integrity of the MFs. The data indicated that MFs may not be involved in the graviresponse of maize roots.

A HGMF, [dynamic factor $(H^2/2) = 1.2 \times 10^{10} \text{ Oe}^2/\text{cm}$] was used to measure the magnetic susceptibility of amyloplasts by magnetograviphoresis. The susceptibility varied between -7.9 to $-8.2 \times 10^{-7} \text{ emu}$ and was close to that of starch. Amyloplast density (estimated by isopycnic centrifugation in metrizamide) ranged from 1.36 to 1.38 g/cm^3 . Magnetograviphoresis was sensitive enough to detect a reduction in the starch content of amyloplasts in light-deprived seedlings.

Physiological studies of the effect of HGMF (dynamic factor $H^2/2$ of 10^9 to $10^{10} \text{ Oe}^2/\text{cm}$) on coleoptiles and hypocotyls rotated on a 1-rpm clinostat showed curvature similar to that induced by gravity and the cells affected by HGMF showed clear intracellular displacement of amyloplasts. The small size of the area of non-uniformity of the HGMF allowed mapping of the sensitivity of coleoptiles by varying the initial position of the wedge relative to the coleoptile apex and showed that below one mm from the tip, only half of the coleoptiles curved toward the wedge indicating that the cells most sensitive to intracellular displacement of amyloplasts and thus gravity sensing are confined to the top one mm portion of (barley) coleoptiles. Similar experiments with hypocotyls (*Lycopersicon esculentum*) also resulted in curvature toward the HGMF. The data strongly support the amyloplast-based gravity sensing system in higher plants and the usefulness of HGMF to substitute gravity in shoots.

The application of high gradient magnetic fields to investigate the gravity-sensing mechanism of plants has wide implications on two levels. First, the research utilizes and improves a novel mechanism of intracellular displacement of starch-filled amyloplasts and the resulting growth response of plants. Second, the growth response is likely to be tightly linked to the perception of a stimulus analogous to gravity. Therefore, the research addresses the larger problem of studying the signal perception/response mechanism in plants. Such studies will generally promote our understanding of plant growth regulation. In particular, the high gradient magnetic field-dependent growth response will elucidate the change in elongation growth and thus directional deposition of cell wall material biomass. In addition, as with all basic research, an improved understanding of basic growth phenomena will have important implications for improving growth and biomass production on Earth, and for better understanding of the bio-mechanic properties of growing plants.

FY97 Publications, Presentations, and Other Accomplishments:

Baker, R.P., Zavada, M., and Hasenstein, K.H. "Hormonal changes after compatible and incompatible pollination in *Theobromacacao* L." HortScience (In Press).

Baluska, F. and Hasenstein, K.H. "Root cytoskeleton: Its role in perception of and response to gravity." *Planta*, 203, S69-S78 (1997).

Blancaflor, E. and Hasenstein, K.H. "The organization of the actin cytoskeleton in vertical and graviresponding primary roots of maize." *Plant Physiol.*, 113, 1447-1455 (In Press).

Hasenstein, K.H., Kuznetsov, O.A., and Blancaflor, E.B. "Induction of plant curvature by magnetophoresis and cytoskeletal changes during root graviresponse." *Proc. 6th Europ. Symp. In Life Sci. Res. In Space, ESA SP-390*: 71-74.

Kuznetsov, O.A. and Hasenstein, K.H. "Magnetophoretic induction of curvature in coleoptiles and hypocotyls." *J. Exp. Bot.* (In Press).

Kuznetsov, O.A. and Hasenstein, K.H. "Ponderomotive magnetic forces can substitute gravity for plants." *ASGSB Bull.*, 10, 55 (1996).

Kuznetsov, O.A. and Hasenstein, K.H. "The response of the lazy-2 tomato mutant to HGMF and red light." *Plant Physiol.*, 114, S284.

Kuznetsov, O.A. and Hasenstein, K.H. "Magnetophoretic characterization of the plant gravity receptor." *Proceedings of the First International Conference on Scientific and Clinical Applications of Magnetic Carriers* (Rostock, Germany). Häfeli, U., Schütt, W., Teller, J., and Zborowski, M. eds; Plenum Press: New York, p 429-444.

Wan, Y. and Hasenstein, K.H. "Purification and identification of ABA binding proteins and antibody preparation." *J. Mol. Recognition*, 9, 722-727 (1997).

Ca²⁺ Metabolism and Vascular Function After Space Flight

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Co-Investigators:

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Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-01

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$165,528

Flight Information:

Flight Assignment: NIH-R4 (STS-85, 1997)

Responsible NASA Center: Ames Research Center

Task Description:

Deficits in calcium intake are associated with increased blood pressure, decreased bone mineralization, and impaired calcium metabolism. Calcium losses due to exposure to microgravity may result in a similar constellation of outcomes. To test that hypothesis, fourteen 7-week-old, male spontaneously hypertensive rats (SHR) were flown on STS-80, an 18-day shuttle mission. Beginning at 3 weeks of age, half the rats were fed a low calcium diet (0.2%) and the other half were fed a high calcium diet (2.0%) diet. The animals were maintained on the diets throughout the experiment. Preliminary results indicate that systolic blood pressure, measured in conscious SHR 3 hours after landing using an indirect tail cuff method, was somewhat lower in the flight animals relative to concurrent ground controls ($p = .053$). When anesthetized with halothane (2% in O₂) just prior to catheterization for blood sampling, direct arterial blood pressure was found to be significantly higher ($p > .0001$) in the flight animals than the control animals in both diet groups (+18 mmHg on average). The differences in blood pressure may have been related to variations in vascular smooth muscle function. Mesenteric resistance vessels from flight animals had smaller maximal contractions to norepinephrine than control animals ($p < .0001$) and showed poorer relaxation to acetylcholine, calcium and sodium nitroprusside ($p < .0001$). Ionized calcium values between diet groups were much closer together in the flight animals (1.34 vs. 1.38 mmol/L, $p < .01$) than the controls (1.24 vs. 1.36 mmol/L, $p < .001$). Parathyroid hormone values for flight animals were 198 vs. 127 pg/ml for the low and high calcium groups respectively ($p < .05$). Vivarium control values were 145 vs. 46 pg/ml ($p < .001$). These values indicate that microgravity increased PTH levels while preserving the dietary difference. Basal free intracellular calcium values were decreased in platelets from flight animals ($p < .05$) while thrombin and ionomycin stimulated calcium levels did not differ from control animals. Finally, animals on low calcium diets were reported by the crew to be more active in orbit. Ground observations confirmed their report and found that the low calcium vivarium controls were more active as well. Overall, the preliminary data indicate that exposure to microgravity had a profound effect on blood pressure

regulation, vascular function, calcium metabolism, and activity. As we continue our assays and analyses, additional observations and insights will be forthcoming.

The principle aim of NIH.R4 was to determine the influence of dietary calcium and microgravity on calcium metabolism, blood pressure regulation, and vascular function in the SHR. Beginning at 3 weeks of age and continuing throughout the experiment, the SHR were maintained on either high (2.0%) or low (0.2%) calcium diets. At 7 weeks of age, the two diet groups ($n = 7/\text{diet}$) were flown on the Space Shuttle Columbia. Two ground control groups were included in the experiments completed at Kennedy Space Center. One was a genetic control for the spontaneously hypertensive rat, the Wistar-Kyoto rat, and the other was a maturational control. Both control groups were fed diets identical to the flight group.

On recovery from the space shuttle, conscious blood pressure measurements were completed within 6 hours of landing using an indirect tail-cuff method. Direct recordings of mean arterial pressure were subsequently made from the carotid artery while the animals were anesthetized with halothane (2% in O_2) prior to blood sampling. Direct blood pressure measurements were completed within 17 hours of landing. A number of additional end points were measured on each of the rats including mesenteric resistance artery function, platelet intracellular calcium at rest and following agonist stimulation, serum levels of calcium regulating hormones, whole blood ionized calcium and serum electrolytes, bone mineralization, and calcium binding proteins (calmodulin and calbindin D9K).

The results show that blood pressure was altered by both diet and microgravity but there was no interaction between the two variables. Blood pressure was significantly higher in animals on low calcium diets than animals on high calcium diets throughout the experiments. Blood pressure was somewhat lower in the conscious flight animals after landing than in the SHR control group ($p = .053$). However, after being anesthetized, blood pressure in the flight group was, on average, 18 mmHg higher than in the SHR control group ($p < .0001$) regardless of dietary condition.

As with blood pressure, there were substantial changes in vascular function in the flight animals independent of the dietary condition. Overall, the mesenteric vasculature was less responsive in the flight animals than the control animals to all stimuli. There was less contraction to norepinephrine and less relaxation to acetylcholine. There was no difference in the response to sodium nitroprusside. The net result of these changes on total peripheral resistance are unknown. While it might be assumed that the reduction in maximal contraction to norepinephrine would be indicative of a probable reduction in vascular resistance, the apparent dysregulation of endothelial function and the subsequent ability to relax the vessels to the extent observed in normal animals argues in the other direction. That is, the vascular response to the many vasodilatory signals that impinge on the vessel may be more important in determining vascular tone than the response to norepinephrine. Impaired vascular relaxation would lead to an elevation of total peripheral resistance and may have contributed to the shift in blood pressure regulation in the flight animals. The presence of significant correlations between mean arterial pressure and vasodilation in our data but not between norepinephrine induced-contractions and mean arterial pressure is indicative of the importance of vascular relaxation in determining blood pressure.

The vascular data are of paramount importance to the issue of orthostatic intolerance. Failure of the vasculature to respond appropriately to either vasoconstrictors or vasodilators limits the potential for hemodynamic adjustments and may underlie, in part, the orthostatic intolerance that has been reported in astronauts on return to Earth's gravity. Limited vascular responses may be inadequate to compensate for the reductions in stroke volume that occur post space flight.

Along with the changes in blood pressure regulation and vascular function, there were alterations in subcellular calcium handling. Basal platelet intracellular free calcium was higher in animals on low calcium diets. Likewise, calcium storage, as indicated by peak calcium release to the calcium ionophore ionomycin, was elevated in animals fed low calcium diets. Space flight animals had lower levels of basal intracellular free calcium. Intracellular calcium levels were closely associated with vascular relaxation.

Systemic calcium metabolism was also altered by space flight. Whole blood ionized calcium was predictably elevated by microgravity ($p < .001$). Parathyroid hormone was elevated in the space flight rats but $1,25(\text{OH})_2\text{D}_3$ was not elevated and calcitonin was reduced. The changes related to microgravity conditions were comparable across diet groups such that differences that existed on the diets in the ground controls were apparent in the flight rats as well.

With regard to bone mineralization, the data indicates there was remodeling of the skeleton. There was significant accretion of bone in the skull of the flight animals while bone mineral density was decreased in the femur. Supplemental dietary calcium resulted in increased bone mineral density throughout the skeleton and thus provided greater protection against losses due to microgravity. There was no significant interaction between calcium intake and space flight in the DEXA bone data. However, closer inspection of the femur and tibia using histomorphometric techniques indicates there were interactions between diet and space flight in terms of the pattern of bone turnover. Further analysis is needed to integrate these findings.

To summarize the results, we have gathered important new information that suggests a change in blood pressure regulation following space flight and the change in regulation may be related to alterations in vascular function.

The research conducted on PARE-04 will benefit mankind in multiple ways. First and foremost, the research addresses the issue of essential hypertension, a disease that afflicts 40 million Americans and is a leading risk factor for strokes and heart attacks. In the past 10 years it has become apparent that calcium metabolism is closely linked to blood pressure regulation. In part, the link may be due to the role of calcium as a second messenger within cells. One manifestation of that role is increased vascular tone when intracellular calcium levels are high. Paradoxically, increasing the level of calcium available outside of the cell through increased dietary calcium reduces calcium levels inside the cell. This promotes vasorelaxation and lowers blood pressure. Understanding the pathways that link calcium as a nutrient that is ingested to calcium as a second messenger will help us understand the etiology of hypertension. The research that will be undertaken in PARE-04 will provide new perspectives on the role of calcium in blood pressure regulation. Zero gravity conditions place considerable strain on calcium metabolism because of unloading of the skeleton and loss of bone calcium. In the short term, resorption of calcium from the skeleton results in hypercalcemia, altered calcium regulating hormones, and reduced absorption of calcium. Ultimately, it leads to a depletion of calcium stores that may result in elevated blood pressure, osteoporosis, and other maladies associated with limited calcium availability.

In a sense, space flight is analogous to pregnancy where there is also a drain on calcium reserves as the fetus develops. During pregnancy, blood pressure is particularly sensitive to dietary calcium intake. Low levels of dietary intake are associated with elevated blood pressure and the development of gestational hypertension and preeclampsia. Supplemental dietary calcium, on the other hand, lowers blood pressure and reduces the risk of developing a hypertension disorder during pregnancy by two-thirds. Dietary calcium supplementation may prove to have a beneficial effect during space flight as well. The issue of dietary calcium and blood pressure regulation is one that affects all of us. The more we understand about the biological processes relating calcium intake to blood pressure regulation, the better we can deal with the issue of hypertension. Zero gravity presents a unique opportunity to explore these relationships in ways that will have benefits for future astronauts as well as for those that remain Earth-bound.

FY97 Publications, Presentations, and Other Accomplishments:

Hatton, D., McCarron, D., Yue, Q., Roulet, C., Xue, H., Otsuka, K., Chapman, J., Phanouvong, T., Roulet, J., Watanabe, M., Nilan, K., Haight, V., Dierickx, J., and Demerritt, J. "Dietary calcium in space." 12th Man in Space Symposium: The Future of Humans in Space, Washington, DC, pg. 80 (June 8 - 13, 1997).

Otsuka, K., Watanabe, M., Yue, Q., McCarron, D.A., and Hatton, D. "Dietary calcium attenuates platelet aggregation and intracellular Ca^{2+} mobilization in spontaneously hypertensive rats." *Am. J. Hypertens.* (In Press).

*Effect of Gravity on the Attachment of Tendon to Bone***Principal Investigator:**

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Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303021

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Task Description:

The strength of the attachment of tendons to bone is important to the movement of the legs. There is little information about the effects of space flight on the attachment of tendons to bone. This experiment is designed to determine if these attachments become weakened during space flight. If so, tendons could be torn from the bone, producing serious injury and pain, thus preventing normal movement of the legs.

This experiment will study the attachment of tendons to the shin bone and heel of rats following their return from space flight. The attachments of the quadriceps and hamstring muscles to the shin bone, and the calf muscle to the heel (the Achilles tendon), will be given special attention. This study will provide new and important information concerning the probability of damage to the attachment of tendon to bone during space flight and will aid in research designed to prevent such injuries to astronauts during future space flights.

This project was concluded during FY97.

Osteoporosis is a disease which affects many people. There has been a debate for many years concerning factors which might cause osteoporosis. Many people feel that inactivity may be a primary cause of the disease. Space flight is an excellent way to produce bone inactivity, as the bones receive no load in microgravity. In this study, all space flight animals developed osteoporosis, suggesting that space flight could be a factor in development of osteoporosis if the flight was lengthy. Since rat bone is similar to human bone but a rat's

metabolism is much more rapid than that of a human, if the osteoporosis occurred in rats during a 11 day flight, it could occur in humans experiencing a longer (3 month) space flight. There was evidence of microfractures in the tibia of space flight rats, suggesting that bones weakened by osteoporosis during space flight may fracture on return to Earth. This event could disable astronauts on their return to gravity. The results of this study also suggested that loading bones weakened by osteoporosis will not promote healing, but will likely result in fracture.

FY97 Publications, Presentations, and Other Accomplishments:

Johnson, R. B., and Abel, S. E. "Comparison of effects of spaceflight on hindlimb and forelimb bones." (Abstract) ASGSB Bull., 10, 59 (1996).

Plant Embryos and Fidelity of Cell Division in Space

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Solicitation: 93-OLMSA-05

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Expiration: not available

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Flight Information:

Experiment ID: 9305010

Flight Assignment: BRIC-08 (STS-78, July 1996)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC

Task Description:

The study tested if the cell division changes observed in the daylily embryo cultures and plantlets result directly from microgravity or indirectly through water availability. Preliminary results from STS-47 and STS-65 have shown genetic abnormalities occur in plants during space flight. Because ground-based studies indicate that water related activity can impact the integrity of chromosomes, it is possible that the results observed on these flights are not due to direct effects upon the plants, but are indirect effects mediated by water availability to plant cells.

Details:

- BRIC 100 canisters housed 27 petri dishes of daylily cells in an agar type medium;
 - There was no inflight manipulation;
 - Upon landing, 85% of the cells were chemically fixed for examination while 15% were allowed to develop;
- and
- Ground controls were allowed to develop in parallel to the flight experiment.

Hypothesis: We have proposed that the chromosomal and nuclear abnormalities encountered in various plants exposed to space are due to a combination of factors including the biological status of the systems and the way in which they are grown, exposed to, and ultimately, the way in which they experience multiple stresses. The central idea is that the extent to which space-specific changes become manifest is dependent on the extent of pre-existing stresses in the system. This has been suggested in a variety of plant species grown in space but has been particularly amenable to study using our *in vitro* developing daylily embryoid system. The following summary hypothesis based on flight observations allows us to harmonize disparate results from several space experiments: (a) the more completely developed a system, the less likely it is to show cell stress during growth; the less morphologically complex, the greater the vulnerability; (b) the "size/packaging" of the genome (karyotype) are also significant experimental variables; plants with larger genomes (e.g., polyploids) seem to be

more space-stress tolerant; and (c) a single space-associated stress is inadequate to produce a significant adverse response unless the stress is severe or a biological parameter necessary to “amplify” it exists. On this view, an appropriate “stress match” with other non-equilibrium determinants, much like a “tug of war,” can result in genomic insults being manifested in space-grown materials.

The general questions being asked were: Can altered mitosis and chromosome behavior in developing plant cells predictably be adversely modified by the space environment by modifying the experimental set-up to deliberately achieve a non-optimized environment? The more specific question is: Can adverse alterations in osmotic status and water relations (water stress) pre-dispose cells to become damaged cytologically in the space environment? Some of the answers are now available. Embryogenic plant cells developing in space under water stress conditions actually seem to do better than those that have excess water. It appears that a slight lack of water (i.e., water stress or drying) allows growth to proceed in a more normal mode. Cells that are more “wet” do more poorly in terms of their morphology and their chromosomal integrity.

Findings to Date

The embryogenic cells of daylily in the plastic petri dishes in the BRIC canisters survived the flight well and were able to grow adequately in the period of the flight -- that is the cells were able to develop and progress through the various stages of somatic embryogenesis. Samples that were on petri dish surfaces that were adjusted osmotically to be more wet did more poorly than those that were on membrane and activated charcoal-impregnated filter paper. This latter device was utilized to enable a more drying environment to be achieved. The essential mineral and organic nutrient components of the medium were the same. Thus, differences were not nutritional in the strict sense but only in terms of differences in the environment through which the nutrient was gained. That is, “more wet versus more dry,” and in terms of the degree of advancement of the embryo initials that were exposed, namely “less developed and more developed.”

From the inception of the experiment, the duration of the experiment was not expected to adversely affect the overall viability of the daylily test materials. The embryogenic initials of daylily are best and certainly most precisely viewed as very young embryos, even “zygote-equivalents.” They are like developing fertilized egg cells or zygotes except they are not the products of a sexual union. They are not undifferentiated cells that need to be induced to form somatic embryos. This may seem like a subtle point but it is in fact a major one for our experiment. The quality of somatic embryo advancement is dependent upon a proper phenotype (what they look like) and genotype (their genetic fidelity or ‘closeness to type’ in terms of what they started off as).

Unlike the cells we used in the experiment, undifferentiated cultured cells are much more in need of careful tending and continuous nutrition. If there are major perturbations such as depletion of endogenous nutrient reserves, or if the nutrients in the exogenous medium are depleted, they deteriorate and die. The embryo initials that we used, on the other hand, are much less in need of continued nutrient. Somatic embryos cease their development and enter into a dormancy when nutrient is exhausted, provided the embryos have grown to a certain level of maturity. (In that regard they are like the germ or embryo of a sexually-derived seed).

The asepsis or sterility of the cultures were maintained for the duration of the experiment. The daylily somatic embryos were completely aseptic at the outset of the experiment. Strict procedures of asepsis were used in setting up the cultures and the material was monitored before the experiment was set up. The individual culture dishes were sealed with parafilm and were thus closed from the ‘outside world.’ The materials returned virtually completely aseptic.

The canisters were flushed with gases (nitrogen, oxygen, and carbon dioxide---75%, 20%, and 5% respectively) and then the canisters were sealed shut so there is nominally no, or at least minimal leakage. Thus the materials were contained inside a more or less clean environment. Since the nutrient medium used was a simple one (it contains only sucrose and thiamine hydrochloride as ‘organics’), no fastidious micro-organisms or pathogens were expected to be able to grow. In what we thought would be the highly unlikely event a dish became contaminated, its growth was to have been limited by the availability of carbon source (sucrose) and, moreover, the contaminant would be isolated in the sealed dish. Since the gases turned out to be more “drying” than had been expected, many embryo units were upon closer examination, considerably desiccated due to the duration of

the experiment, and the apparent stressful conditions of the flight (more later). There was plenty of volume of medium present in the flight petri dishes from which nutrient could be derived, if conditions permitted. Growth has been initiated from a great many of the "space-grown" units. And scanning electron microscopy has been performed on representative samples from each of the flight and ground control categories.

The main cytological observation that was made involved the detection, once again, of a so-called deletion. A substantial part of a chromosome is missing in all the cells of the flight samples that were more "wet." However, deleted forms were also found among those embryoids that were "more dry" as well. The prediction was that we would find more deletions in the "wet" and fewer in the "dry." The fact is, however, that the deletions were present in both wet and dry. No ground control material showed the deletion. As predicted, there were more signs of chromosome perturbations in the "wet" than the "dry" (5.3% versus 0.6%). And again as predicted, chromosomal perturbations were found more in the "wet" than in the "dry" (3.3% versus 1.1%). The larger, more advanced somatic embryos showed fewer and less severe chromosomal perturbations than the smaller and less developmentally advanced embryoids (0.8% versus 2.3%). Thus, the main predictions were borne out except for the important and significant deletion of the chromosomes in all samples. All forms of embryoids in space showed the deletion. The hypothesis is only partly correct apparently. This leads us to speculate that the deletion is probably the most significant of cytological indicators of stress in daylily in the space environment, and is able to over-ride all other signs of stress, such as chromosomal damage and aberrations of cell division, etc. Size of the unit undergoing the stress may be significant in terms of more transient and less significant damage such as breaks and laggards, bridges, and micro-chromosomes, but it was not enough to offset the production of a deletion. Since the deletion has been consistent in all materials from various spaceflights, it may well be that there is an active site that responds to the spaceflight environment.

Another task has been to carry out an extended duration experiment on Mir. NASA's Shuttle 'Atlantis' (Space Transportation System, STS-81), had a perfect lift-off from Cape Canaveral, Florida on Sunday January 12, at 4:27 a.m. EDT for a 12 day flight. "Link-up" with the Mir Space Station occurred on Wednesday January 15. On 17 January 1997 the experiment was transferred in the morning shift to the 'Piroda' module of Mir. Separation of the Mir from the Shuttle occurred on Sunday 19 January 1997 at 9:15 p.m. EST. Landing of Atlantis occurred at Kennedy Space Center on January 23. Atlantis had paid its fifth visit to 'Mir'. The control material from our experiment package at Hangar L was in the OES until word of the transfer occurred and then moved to the caged area of the Hangar. That way it would not risk damage from chambers over-heating or whatever for the duration of the lengthy experiment. More importantly, the fluctuations of the hangar were expected to more normally approximate the environment of the Mir.

One of the very interesting results that we have obtained thus far from the Mir materials is the change in phenotype of those embryo stages that had grown sufficient to show distinct leaf morphology. We established in this laboratory years ago that 1 microliter of gaseous ethylene per liter of "air" in sealed vessels fosters transition of daylily leaf arrangement from "juvenile" to the "mature" phenotype. *Hemerocallis* "seedlings" are rather spindly (superficially resembling a green onion or scallion) and although their leaf arrangement is distichous, or two-ranked, the fan-like habit of non-juvenile daylilies only appears after several months' growth. Under field or greenhouse conditions in temperate climates, this occurs towards the end of the first growing season. [Smith, D.L., K. Kelly and A.D. Krikorian, 1989. Ethylene-associated phase change from juvenile to mature phenotype of daylily (*Hemerocallis*) *in vitro*. 76: 466-473.] The Mir flight material which had grown from stages sufficiently advanced to yield good leaf development, showed fan-like leaf arrangement. Ground controls showed little fan-shape development. Clearly, the space environment of Mir had more units with fan shapes than those in the control group. Based on our previous ground work, the involvement of ethylene for the fan-shape seems apparent and that is being pursued in greater depth in laboratory studies in place now. The significant point is that the fan shape phenotype is, as experienced on Earth, not fixed. New growth is juvenile. Examination of root material from the mature forms does not show chromosome breakage and damage. The materials do show, however, that the deletion is present, and has been shown in all the materials. In short, it does not appear in the Mir experiment, as indeed it was in other experiments on Earth and in space, as if ethylene is responsible for any of our previously encountered chromosome damage. Ethylene has never been shown by us to cause chromosome breakage.

Materials were fixed for scanning electron microscopy (SEM) by subjecting to 2.5% glutaraldehyde and 1% formaldehyde (buffered in .05 M Sorensen's phosphate buffer pH 6.8) prepared from paraformaldehyde powder for two hours at 4°C. Following this, the samples were fixed for 24 hours at 4 C in 2.5% phosphate buffered glutaraldehyde. The samples were post-fixed in 1% osmium tetroxide for 2 hours at 4°C. After dehydration, through graded ethanol series, the samples were critical point dried and coated with gold. The specimens have been examined, and are still being examined in a JEOL JSM-5300 Scanning Electron Microscope using Vital Image Technology software.

One question is whether there are other substances or features of the space environment potentially responsible for the fan-habit? Experiments carried out to examine any possible role of temperature and light in the initiation of the fan habit showed years ago that these factors are not responsible for the transition from juvenile to mature phenotype (Fitter and Krikorian, 1985). Also, the hypothesis that "maturity-inducing/sustaining" substance(s) are produced and reduced into the medium by fan plantlets, and that mature growth is thus prompted in juvenile plants, was tested in this laboratory and shown not to be tenable. When juvenile forms were placed aseptically on media in containers from which fan plantlets were removed, and vice versa, the juvenile forms remained juvenile and the fan forms remained juvenile and the fan forms soon produced new juvenile growth. Juvenile forms do not grow into fan forms under *in vitro* conditions – even when a substantial period of time has elapsed and one might normally expect fans. This may perhaps may be due to depletion of nutrients and the dramatic slowing down of growth in cultures which have been maintained for many months in the same medium (Fitter and Krikorian 1985). [Fitter, M.S. and Krikorian, A.D. 1985. Mature phenotype in *Hemerocallis* plantlets fortuitously generated *in vitro*. *Journal of Plant Physiology* 121: 97-101.]

The significance of the work is that we have demonstrated that daylily embryos can last a significant period of time in a space station and retain their viability. They can resume normal growth after they have reached maturity on a single 'feeding' petri dish and are then transferred to a fresh nutrient medium. The fresh medium used is designed to cause them to sprout. The somatic embryos begin to grow and continue to developmentally advance into plantlets in a very short time, a matter of a few days. In short, based on our prior work and experience with the system, there was every reason to believe that the materials would survive the extended exposure on Mir. The finding is significant in several contexts. Perhaps the most important is that there is a bona fide potential to store biological materials in an "inert" but viable state for long-duration missions. The scenario would be that stored embryoids would be resuscitated, grown, and utilized in a CELSS-type setting or for experimentation. Additional Post Flight Analysis is in progress. Material recovered is being examined as promptly as possible consistent with reliable collection of data. Examinations began as promptly as possible after recovery. As anticipated, some materials were fixed directly upon recovery. Other recovered materials were transferred to fresh media, and then sampled over a period of days to ascertain chromosomal status. Still other samples are being reared to later stages and examined. Features analyzed, therefore, will include gross morphology, general cellular morphology, chromosome morphology, and continued growth and adaptation post-flight.

Plants are important from many viewpoints. From the time we get up in the morning and brush our teeth with toothpaste thickened with plant extracts and flavored with peppermint oil, to the food we eat, to the garments we wear, to much of the furniture we use, to the cotton pillow and sheets we lie down on at bedtime, our daily lives are intimately affected by plants.

Throughout the complex process of plant growth, a series of well-orchestrated and coordinated events ranging from cell division to differentiation must occur. Indeed, under field conditions, if the needed cell biological and biochemical and molecular events that ultimately give rise to resultant form and function are not properly mobilized and realized both temporally and spatially, the plant will lose out in the competition with better "designed" plants. At the laboratory level, major differences in structure, metabolism, biochemistry, and ultrastructural architecture will be apparent. There is strong evidence that gravity plays a major role in directing the way in which plant cells "orchestrate" these required events in their zones of cell division, differentiation, and maturation. Developing somatic embryos of daylily, *Hemerocallis*, provides an excellent model system that will allow us to test how the cell biological, biochemical, and physical events leading to the formation of both

specialized and unspecialized types of cells comprising the plant embryo are uncoupled, or mobilized and modulated during growth in a long-duration microgravity environment. An experiment has been designed to provide detailed insights into these important processes by examining growth and cytological and biochemical performance in daylily embryocytes in specially contrived configurations designed to reveal special responses at the level of morphology, histology, cell biology, ultrastructure, biochemistry, and chemistry. Development and responses in protracted microgravity will be looked at from the perspective of the level and precision of cell division in critical growing regions of embryo shoot and root apex as they respond to alterations in their loading-bearing capabilities, mitotic errors that might occur during differentiation and specialized cell and tissue development in the novel environment of microgravity, and the level of activity of the genome in terms of special gene expression in expected and unexpected ways. Efforts will be made to characterize changes in the cell cycle and potential modifications to it as a consequence of adaptation or response to development in microgravity as compared to normal development at 1-G. Any changes detected in altered growth characteristics or disturbed development in microgravity will be correlated at the cell, tissue, and organ levels and further detailed at the biochemical and molecular levels. Application of a series of cell biological and biochemical techniques will be brought to bear to explain the mechanism(s) of responses that we expect to detect at the level of cells, tissues, organs, and the entire embryo. A major effort will be directed towards determining unequivocally whether observed perturbations to the daylily system are due to microgravity proper or whether they are due to indirect effects of the space environment. Accordingly, this project recognizes that in order to obtain reliable data in this important experiment, considerable effort has to be expended through pre-flight ground studies to ensure that as rigorously controlled an environment will be attainable for the performance of the experiment.

To the experimental biologist, plants are very interesting since they have evolved mechanisms that enable them to sense and use various environmental signals and messages to their advantage in the course of their lives. Because plants are generally immobile, they have to deal with situations as they arise—they cannot "run away." Plants are very well adapted to using "information" from the outside world. This means that an understanding of how plant growth control is achieved is very important.

Space flight experiments show that metabolism, productivity, and specialization characteristics of a variety of plant cells are altered. The study of these reactions to space has led to a better understanding of the ways plant cells, especially cultured embryogenic cells, grow and the mechanisms by which such cells develop and control production of cell components which are important in agriculture.

More specifically, our research has been concerned with how growth and development of embryos and plantlets from non-sexual (body) cells is affected and controlled by gravity and the space environment. Cloning experiments have been carried out on Earth and in space to generate embryos from embryocytes (also known as embryo initials) and the work indicates that there is significant impact on embryo differentiation and growth. Studies on the nature of shape and form, genetic changes and how they occur and are modulated has shed significant light on the process of regeneration from cultured plant cells. This information is critical to much plant biotechnology since the field relies on manipulating, changing, and managing developing plant cells and regenerating and cloning plantlets from them. One of the current and major constraints to reliably controlling genetic engineering in plants for overall improvement purposes is the lack of a full understanding of the controls mechanisms as free cells develop into embryos and from these plants. The information gained from Earth and in space has pointed the way to exploring the control mechanisms more effectively.

It is clear that the intimate and adaptive, even evolutionarily controlled, relations between the atmosphere, the soil, and the growing plant that are normally achieved on Earth will not be easily duplicated in space. A somewhat empirical approach appears justified based on our limited knowledge of space flight environments and the responses of plants in those environments. Clearly more experimental data need to be obtained under well-defined conditions.

In this context, it is important to recognize that modern plant science and agricultural engineering have produced some remarkable advances, but none of these would have been possible without the ability to build on an ancient foundation. A similar foundation is not available for those wishing to grow plants in space. We therefore will only be able to make progress if we have a body of data on which we can build.

As real progress is made towards adding reliable baseline data to our store of knowledge, and the growing of plants in space becomes increasingly reliable, it is certain that many members of the scientific community will become attracted to carrying out space biology experiments. As it now stands, limitations in our ability to grow plant materials reliably have prevented a broad plant biological sciences community from becoming more involved. I believe that it is in the best interest of science and NASA not to minimize the constraints to growing plants reliably in space.

FY97 Publications, Presentations, and Other Accomplishments:

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Effects of Gravity on Bone Matrix Production and Mineralization

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-04

Initial Funding Date: 1988

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9304021

Flight Assignment: NIH-C1 (STS-59, April 1994) and NIH-C3 (STS-63, February 1995)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: STL-A and FPA

Task Description:

In humans and other vertebrates, the weightless environment of space flight causes defective skeletal growth, marked by a loss of bone mass and a change toward lower bone maturity. The development of defective bone is believed to involve matrix production controlled by bone cells, bone mineralization, or an interaction between bone matrix production and bone mineralization.

The investigators will use established cell lines of chicken osteoblasts in the Space Tissue Loss (STL) module and Fluid Processing Apparatus (FPA) hardware. The investigators will analyze rates of cell growth, aspects of collagen and bone development, and mineralization outside the cultured cells. Data obtained in the flight experiments should provide knowledge on the effects of gravity on osteoblast activity and function, protein development, and mineralization. The studies will have implications for long-duration space flight, as well as application to the diagnosis and treatment of prolonged skeletal immobilization or mineral abnormalities.

Studies during FY97 have been concerned with examining cultured osteoblasts from embryonic chickens in terms of their phenotype, growth, and development during shuttle flights STS-63 and STS-77. During the former, space flight effects on the bone cells were characterized through the detection of non-collagenous proteins identified by immunocytochemistry of STL-A cartridge contents following this 8-day mission. Three important bone proteins, osteopontin, bone sialoprotein, and osteocalcin, were found in the extracellular matrices from flight cartridges but with reduced immunoreactivity compared to that in control cartridges (maintained at 1-G for the duration of the flight) and basal cartridges (maintained at 1-G until the time of shuttle launch). The appearance of these non-collagenous proteins, as well as type I collagen observed from previous work, was

consistent with earlier results demonstrating the constituents biochemically and an apparent down-regulation of collagen and osteocalcin gene expression in space flight. These data suggest that space flight, and environmental or mechanical forces in general, mediates adaptation of skeletal structure by means of changes in proteins critical to events in bone formation and/or resorption.

Related results were observed with the same system of cultured embryonic bone cells flown aboard STS-77. In FPA hardware utilized during this mission, cells examined 3 hours and 3 days after launch demonstrated by both scanning and transmission electron microscopy changes in their number, phenotype, and substrate attachment compared to cells maintained at 1-G in identical FPA devices. The fewer cells having unusual shapes, more vacuolated cytoplasm, and a highly varied, rather than more uniform, FPA substrate disposition found following space flight compared to normal gravity suggest that the launch environment and/or low gravity conditions resulted in rapid changes in the bone cells. These data, like those noted in STS-63, have significant implications in understanding bone loss effects experienced by humans and other vertebrates under variations in mechanical and gravitational forces.

These experiments measuring the responses of cultured bone cells to space flight and microgravity are extremely useful for describing the manner in which the cells function and adapt to a changing environment. Since the cells are critical for the proper maintenance of the skeleton as a whole, these data are also fundamental for understanding how this principal structural support of the body is controlled. The absence of gravitational force in space (unloading the skeleton) is known to exert profound effects on bone and certain other tissues. The results from these studies, then, may provide insight into the observations that humans lose bone mass during space flight. In addition, the absence of gravity may be correlated with situations on Earth of prolonged bed rest following illness or other examples of human inactivity, failure to exercise, immobilization of limbs during bone repair and healing, and similar conditions. Thus, the data from shuttle experiments may as well generate new knowledge into the reasons for the decrease in bone in these instances on Earth. From the information on bone cell behavior in space flight, it may be possible to develop new approaches to the recognition, treatment, and prevention of bone loss that occurs in man in a variety of circumstances.

FY97 Publications, Presentations, and Other Accomplishments:

Landis, W.J., Hodgens, K.J., Song, M.J., Arena, J., Kiyonaga, S., Marko, M., Owen, C., and McEwen, B.F. "Mineralization of collagen may occur on fibril surfaces: evidence from conventional and high-voltage electron microscopy and three-dimensional imaging." *J. Struct. Biol.*, 117, 24-35 (1996).

Effects of Microgravity on Pathogenesis and Defense Responses in Soybean Tissues

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Co-Investigators:

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Funding:

UPN/Project Identification: not available
 Initial Funding Date: not applicable
 Students Funded Under Research: 4
 FY 1997 Funding: not applicable
 Joint Agency Participation: NSAU

Solicitation: not available
 Expiration: not applicable
 Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9600003
 Flight Assignment: CUE (STS-87, November 1997)
 Responsible NASA Center: Kennedy Space Center
 Flight Hardware Required: BRIC

Task Description:

Anecdotal evidence suggests that plants grown in microgravity are more susceptible to microbial invasion. If true, the implications for crop production in space are serious: the increased accessibility of plants to microorganisms may mean that the plants are not only more susceptible to recognized pathogens, but they may also be susceptible to pathogenic colonization by opportunistic pathogens, i.e., organisms that are not normally pathogens to the plant. The project defined here brings the expertise of molecular and cellular biologists from the Ukraine (O. Nedukha, V. Prima, and E. Kordyum) and the USA (J. Leach and J. Guikema, KSU; C. Brown, KSC) together to explore how microgravity affects plant susceptibility. For the proposed studies, we have selected the interaction between *Phytophthora sojae* and soybean as a model system. *P. sojae* causes root and stem rot of soybean. Our reasons for selecting this disease interaction are the following: (1) *P. sojae* is a devastating pathogen of soybean causing annual yield losses exceeding \$250 million; (2) two of our collaborators (Guikema and Brown) have extensive experience with microgravity-induced alterations in soybean physiology; (3) resistant interactions between *P. sojae* and soybean roots do not require light as do foliar pathogens [in the BRIC hardware where our experiments will take place, there is no light]; (4) the genetics of the interactions between different soybean cultivars and different races of the fungus are well documented; (5) the molecular mechanisms of resistant interactions between *P. sojae* and soybean are well characterized; and (6) the experimental system will provide sufficient amounts of space-grown tissues for the proposed analyses by both Ukrainian and USA scientists.

Our first objective is to quantitatively measure the effects of microgravity on susceptibility of soybean seedlings to root rot caused by *Phytophthora sojae*. Previous observations that plants grown in microgravity are more susceptible than unit gravity-grown plants have not been quantitative. Thus, this objective is important because

as we begin to dissect out which factors contribute to susceptibility and to what extent each contributes, we need an established baseline of quantitative data for microgravity-induced susceptibility.

Increased susceptibility may have several underlying causes; these will be explored in our second objective. Microbial vigor may be increased in microgravity or the microbes may become somewhat resistant to the effects of toxic compounds produced by the plant. Alternatively, changes in the plant cellular structure or the cells ability to respond to pathogens may result from growth in microgravity. In this project, we will emphasize the plant side of the interaction. We will apply concepts derived from the current understanding of how plants resist pathogens, targeting specific flaws that might allow enhanced susceptibility. Enhanced susceptibility in microgravity might result from changes in the preformed plant barriers to pathogen ingress (such as decreased wall strength resulting from reduced lignification) or changes in nutrient partitioning (such as increased carbon availability or membrane fluidity). Our Ukrainian collaborators will compare cell wall structure and plasma membrane structure and fluidity in soybean seedlings grown in microgravity versus unit gravity and determine if these correlate with increased susceptibility to the root rot pathogen, *Phytophthora sojae*. C. Brown will determine if carbohydrate partitioning in the seedlings is altered in microgravity, and if this correlates with susceptibility.

Plant development in microgravity also might impair the plant's ability to actively resist pathogen ingress, that is, induced plant defense compounds may not be formed or, if formed, may not locate to appropriate sites. In general, compounds that are induced during plant resistance responses include soluble antibiotic compounds called phytoalexins, structural reinforcements such as the phenolic polymers lignin or suberin, enzymes such as those involved in phytoalexin biosynthesis or phenolic polymer deposition (e.g., peroxidases), or enzymes that may directly affect the pathogen, such as chitinases or B-glucanases. We will determine if, after growth in microgravity, differences in the accumulation and distribution of structural compounds (phenolic polymers) and enzymes whose accumulation and location is correlated with resistance (anionic peroxidases and phospholipase D) occur in soybean challenged with two races of *P. sojae*, race 1 and 25, which cause resistant and susceptible responses in soybean cultivar Williams 82, respectively, under conditions of unit gravity.

A. Modifications in SOYPAT.

Based on results of the Science Verification Test (SVT) of October 1996, several modifications in the experimental set-up for SOYPAT were implemented. These included seed pouch construction, fungal inoculum application and dosage, days for sampling, and fixation procedures. Seed germination pouches constructed with individual channels for each seed confined roots and prevented tangling of adjacent roots. Fungal inoculum is applied to the bottom of the pouch via flexible tubing; this allows a more even distribution of inoculum and a more even infection pattern.

The sampling days were changed to flight days 3, 6, and 7; the concentration of the fungal inoculum was changed to allow optimal infection on the appropriate sampling times. The original fixation apparatus provided was not approved for flight; new fixation devices were designed based on the requirements of this experiment.

B. Payload Verification Test.

In April-May, 1997, a Payload Verification Test (PVT) was performed at KSC. The changes described above were implemented in this experiment. A major factor that had changed was the use of the new fixation devices (this was the first opportunity to test the devices). Although there were some minor technical difficulties with some individual devices, overall, they worked well and the tissues recovered were in excellent condition.

Plant responses to fungal treatments were consistent with our laboratory studies except that the differences between resistant and susceptible interactions in our laboratory were more definitive than those observed in the PVT. One measure of susceptibility of the soybean tissues to the various strains of the fungus is the effect of infection on root length. Because photographs were not available from some PVT sampling times, we combined data from flight and ground experiments for our quantitative analysis. Since microgravity was not a factor in the PVT, the ground and flight treatments were treated as replications. In the first harvest, seedling

roots were similar in length (ave 2.5 cm), and did not exhibit symptoms of fungal infection. As expected, in later samplings seedling roots from tissues treated with the virulent fungus (susceptible response, 6 day, 4.4 cm ave) were shorter than those treated with the avirulent fungus (resistant, 5.4 cm ave) or the control roots (5.4 cm ave). Analysis by light microscopy revealed that mycelial colonization of the root surfaces occurred in both fungal treatments, but more oospores formed in the susceptible roots than in resistant roots. These data indicate that plant responses are occurring as expected in the BRIC set-up. We are now ready for the flight samples.

The studies proposed will reveal whether growth in microgravity renders soybean plants more susceptible to ingress by a root fungal pathogen, in general, and if microgravity compromises the plant's ability to mount a resistance response. Based on our current understanding of the mechanisms by which plants defend themselves from pathogens and what is currently known about morphological changes in plant cells grown in microgravity, we have predicted a number of potential "flaws" in the host defensive system. The experiments here will provide correlative evidence if some of these predictions are correct or probable. Furthermore, we will gain an understanding of the effects of microgravity on protein distribution in and around plant cells. The comparisons of resistant and susceptible host/pathogen interactions are important because it is possible that impaired development of a performed barrier, such as reduced lignification of the cell wall, may "override" any induced defense responses. Although reasonably well-characterized biochemically, most events associated with defense in unit gravity are correlative. The unique parameter in our experiments as compared to past studies of host plant resistance is the microgravity environment; we predict that comparisons of resistant and susceptible responses in unit versus microgravity will provide new and important clues as to which events correlated with resistance are truly involved in host defenses. Finally, *P. sojae* is a devastating pathogen of soybean, causing annual yield losses exceeding \$250 million; thus, any information that might provide clues to control the disease will be of major benefit to production of the crop.

FY97 Publications, Presentations, and Other Accomplishments:

Chittoor, J., Leach, J.E., and White, F.F. "Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae*." *Mol. Plant-Microbe Interact.*, 10, 861-871 (1997).

Leach, J.E. "Plant defense responses." Seminar presented to the Department of Molecular Biology and Biochemistry, University of Wyoming, Laramie, WY (April 4, 1997).

Effects of Micro-G on Gene Expression in Higher Plants

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10	Solicitation: 95-OLMSA-02
Initial Funding Date: 1996	Expiration: 1999
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$105,849	

Flight Information:

Experiment ID: 9502032
Flight Assignment: BRIC-09 (STS-80); BRIC-10 (STS-85, July 1997)
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: BRIC

Task Description:

Although various effects of microgravity at the whole plant, organ, tissue, and cellular levels have been observed, little is known about the molecular mechanisms involved. The PI proposes to use the BRIC hardware to conduct two experiments which may yield significant results about alterations in gene expression in space-grown seedlings. The first experiment is to use transgenic plants that express GH3 promoter-GUS and SAUR promoter-GUS fusion genes to study the effects of microgravity on expression of auxin- and gravistimulation-inducible genes, GH3 and SAUR, at the organ and tissue levels. This study should not only provide information about how expression of the auxin- and gravistimulation-inducible genes is altered at microgravity as compared to the ground controls (1-G and clinorotation), but also enable us to gain some insights into the effects of microgravity on auxin transport/distribution and/or tissue's sensitivity to auxin in higher plants. The second experiment is to identify and clone genes whose expression is altered under the microgravity environment using a differential cDNA library screening method or RNA Differential Display technique. Molecular cloning and characterization of gravity-regulated genes is a crucial step to elucidate the mechanisms of the gravity effects on growth, developmental, and metabolic processes in higher plants.

Two space-flight experiments were (BRIC-09: November 19, 1996, STS-80; BRIC-10: August 7, 1997) conducted to study the effects of microgravity on the expression of auxin regulated GH3 and SAUR genes in transgenic tomato and tobacco seedlings that express the auxin inducible GH3 gene promoter-GUS and SAUR gene promoter-GUS fusion genes. Our studies indicate that the expression of both the GH3 promoter-GUS and SAUR promoter-GUS fusion genes was significantly enhanced (2 to 4 fold increases) in space-grown seedlings when compared with the ground control seedlings. The activation of the GH3 promoter-GUS fusion gene in tobacco plants was developmental stage dependent, with an activation in cotyledons initially, then in elongating regions of hypocotyls and roots of the space-grown seedlings. As seedlings developed, the activation of the gene was reduced in cotyledons but enhanced in root elongating regions. The activation of the GH3 promoter-GUS gene was relatively higher in vascular tissues and much lower in other tissue types. The expression of the

SAUR promoter-GUS gene in the elongating region of hypocotyls in the space-grown seedlings was also significantly higher than in that of the ground controls. Furthermore, a dramatic reduction in root growth and a significant increase in hypocotyl length were observed in the space-grown seedlings, which is well correlated with the activation patterns of the auxin regulated genes. Currently, the PI and co-workers are further characterizing the effects of microgravity on the expression of the GH3 promoter-GUS and SAUR promoter-GUS genes using microscopic techniques. Also, the PI and co-workers are in the process of isolating mRNA from *Arabidopsis* seedlings from space flight and ground in order to construct a cDNA library and clone genes that are differentially expressed in space-grown seedlings.

Gravity has a variety of effects on the structure and function of plants identifiable at the whole plant, tissue, and cellular levels. The gravitropic response of higher plants makes roots grow downward into soil for absorbing water and mineral nutrients and shoots grow upward for harvesting maximum light energy for photosynthesis which is crucial for maximum productivity of crop plants. Research to examine the effects of microgravity on the expression of auxin-inducible genes (auxin is an important hormone involved in the gravitropic response and growth/development of higher plants) and to clone/characterize gravity-regulated genes should provide some insights into the molecular mechanisms of the plant tropic response and the effects of gravity on plant growth and development. Thus, the research should yield a new understanding of the effects of gravity on higher plants and may provide a basis for improvement of growth rate of higher plants grown in space. The latter is an important step toward the commercial application of space using plants as bio-reactors and the development of bio-regenerative life support systems to support crews in extra-terrestrial environments.

FY97 Publications, Presentations, and Other Accomplishments:

Li, Q., Wu, Y., Hilaire, E., Guikema, J., Brown, C., Piastuch, W., and Li, Y. "Effects of micro-G on expression of auxin-regulated genes." Annual Meetings of American Society of Plant Physiologists, Canadian Society of Plant Physiologists, Australian Society of Plant Physiologists and Japanese Society of Plant Physiologists, Vancouver, Canada (August 1 - 6, 1997).

Osteoblast Adhesion and Phenotype in Microgravity

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Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-04

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$26,313

Joint Agency Participation: NIH

Flight Information:

Experiment ID: 9304022

Flight Assignment: NIH-C4 (STS-69, July 1995); NIH-C6 (STS-80, November 1996)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: CCM

Task Description:

Bone loss during space flight is well documented, but remains incompletely understood. Among the unanswered issues are the direct effects which microgravity exerts on bone cells, and the mechanisms by which these cells recognize changes in gravity. This study will focus on bone cells of the osteoblast family which synthesize bone matrix and may also participate in its breakdown (resorption) by regulating the formation and activity of bone-resorbing cells, osteoclasts. The experiment will test the hypothesis that microgravity can produce direct effects on osteoblastic cells similar to those of regulatory hormones. In addition, the study will examine whether microgravity alters the interaction of osteoblastic cells with their matrix resulting in changes in shape or cellular organization known to affect cell function.

In this study, cells will be cultured in the mid-deck compartment of the space shuttle in the Space Tissue Loss (STL) cell culture module (CCM); parallel control cultures will be maintained on Earth under identical conditions. During the ten day experimental flight period, batches of both control and experimental cells will be fixed for analysis and samples of culture medium will be collected for biochemical studies. Following the flight, the cells will be analyzed to identify changes in shape and function. Medium samples will be analyzed to identify the presence of bone matrix proteins and matrix-degrading enzymes which may participate in early stages of bone change.

Efforts in FY97 focused on repeating the experiment flown on STS-69 with minor modifications. The principal change involved replacement of the collagen-coated Cytodex-3 beads as a substrate for growth of the ROS 17/2.8 osteoblastic cell line with Biosilon beads, which is comprised of tissue culture-treated plastic. This change was initiated to minimize detachment of cells during the experiment, as had been observed in both ground and flight samples of STS-69. The protocols for cell culture and the time course of fixation and medium harvest were unchanged. The second experiment was flown on STS-80 and all samples were recovered successfully.

Evaluation of the cells indicated that both ground and flight samples remained viable and attached throughout the experimental period; however, evaluation of all cultures from the experiment showed evidence of microbial contamination which compromised all data interpretation.

The aims of this research are to determine the effects of microgravity on bone cells in an effort to understand the mechanistic basis for bone loss during space flight. This bone loss due to mechanical unloading does appear to resemble that which occurs on Earth as a result of inactivity (disuse osteoporosis). To the extent that the two conditions involve similar cellular mechanisms, the findings should be applicable.

Microgravity Effects on Pollination and Fertilization

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 4

Post-Doctoral Associates: 2

FY 1997 Funding: not available

Joint Agency Participation: NSAU

Flight Information:

Experiment ID: 9600004

Flight Assignment: CUE (STS-87, November 1997)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: PGF

Task Description:

The conclusion of a third flight experiment on reproductive development in *Arabidopsis* in fall 1994 (STS-68) provided us with extensive material for analysis of seed formation during space flight. These seeds were prepared for microscopy and stained for cytochemical localization of seed storage reserves. When compared with ground controls, the material was found to be indistinguishable. Previous experiments on STS-54 and STS-51 with this same biological system had shown reproductive development to abort during space flight prior to seed formation. These experiments were conducted in closed chambers which probably leads to stagnant air layers around the plants in microgravity where convective air movement is lacking. On STS-68, an active airflow was provided, and all stages of reproductive development occurred normally on orbit. Since reproductive development thus seems to be possible in microgravity if proper environmental conditions are provided, we have sought a new experimental system that will allow us to closely compare the timing of developmental milestones in space flight and ground control material. This was not strictly possible in *Arabidopsis* because of its pollination biology. *Arabidopsis* is self-pollinating. For our new model system, we sought a plant that requires pollen transfer from a different source (i.e., it is self-incompatible) in order to closely control the timing of pollen transfer to the stigma and therefore initiation of the fertilization sequence. *Brassica rapa* has been chosen for this experiment. *Brassica rapa* is closely related to *Arabidopsis*, however it bears larger flowers that can be manually pollinated using a pollination device. Through the Crucifer Genetics Cooperative, many different lines of rapid-cycling *Brassica rapa* are available, and line 1-59 is especially well-suited for space flight experiments because its short stature allows it to fit within the confines of a Plant Growth Chamber in the Plant Growth Facility flight hardware. Details of soil-less culture were worked out to allow an easy integration of 11-day old plants into PGCs prior to launch. In-flight procedures including labeling flowers, pollinating, and in-flight fixation procedures were determined. This effort is geared to the scheduled launch of an experiment on STS-87 as part of the CUE payload in October 1997.

During FY97 we participated in several full duration tests of the CUE experiment design at Kennedy Space Center. While the Science Verification Test went well, the subsequent Payload Verification Test pointed out problems with hardware and payload specialist activity. As a result, our lab provided extensive support for additional hardware tests by providing material for the tests and evaluating the plant material obtained after each test. We also performed numerous crew training sessions in anticipation of the November 1997 flight experiment. Analysis of material obtained from the test runs with light and electron microscopy has verified the fidelity of the procedures to be used on orbit and in the post-flight analysis. Since an education project is running in parallel to this experiment and is based on this experiment, we have provided support for the development of the curricular materials that have been used in the teacher training workshops conducted nationwide. Locally we provided a 2-day training workshop for 50 high school biology teachers. Preparations are currently underway for the actual flight experiment.

Understanding physical processes that contribute to the success or failure of plant reproduction will allow plant biologists to make further progress in tackling problems in plant pollination and fertilization under normal conditions. The microgravity environment of orbital platforms provides a unique mechanism for investigating these physical processes, since gravity-dependent processes are perturbed in ways that are not possible in a 1-G environment.

However, another way this project is of value is in the pride and excitement it stimulates. Interest in the space program is high, especially among children. The PI has found that the goodwill these experiments foster toward basic research in general is extremely important. Growing plants in space is something that excites the average person and heightens awareness of science in general. In 1997 the CUE will be featured in the news and will likely obtain national and international press coverage. This experiment is paired with an educational outreach component, CUE-TSIPS, which provides classroom simulation of the space flight experiment for children in the US and Ukraine. This is an unprecedented attempt to bring public involvement into a plant space biology experiment. When people think about why plants would be grown in space (i.e., to provide food and atmosphere cleansing for the crew members), they may remember and appreciate the importance of agriculture to sustaining their daily lives on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Crispi, M.L., Porterfield, D.M., Murgia, M., and Musgrave, M.E. "Role of metabolic gases in reproductive failure under spaceflight conditions: Ground based studies with *Arabidopsis*." Life Supp. Biosphere Sci. (In Press).

Kuang, A., Crispi, M.L., and Musgrave, M.E. "Control of seed development in *Arabidopsis thaliana* by atmospheric oxygen." Plant, Cell & Environ. (In Press).

Kuang, A., Xiao, Y., and Musgrave, M.E. "Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions." Ann. Bot., 78, 343-351 (1996).

Musgrave, M.E. "Space can't wait." Nature, 388, 823 (1997).

Musgrave, M.E. "Plant reproduction in spaceflight environments." International Space Station Symposium, American Society for Gravitational and Space Biology Annual Meeting, Charlotte, NC (October, 1996).

Musgrave, M.E. "Plant reproduction under spaceflight conditions." Seminar at the NASA Specialized Center for Research and Training in Gravitational Biology, North Carolina State University, Raleigh, NC (July, 1997).

Musgrave, M.E., Kuang, A., and Porterfield, D.M. "Plant reproduction in spaceflight environments." ASGSB Bull., 10, 83-90 (1997).

Musgrave, M.E., Kuang, A., and Matthews, S.W. "Plant reproduction during spaceflight." Planta, 203, S177-S184 (1997).

Porterfield, D.M., Crispi, M.L., and Musgrave, M.E. "Changes in soluble sugar, starch, and alcohol dehydrogenase in *Arabidopsis thaliana* exposed to N₂ diluted atmospheres." *Plant Cell Physiol.* (In Press).

Porterfield, D.M., Matthews, S.W., Daugherty, C.J., and Musgrave, M.E. "Spaceflight exposure effects on transcription, activity and localization of alcohol dehydrogenase in the roots of *Arabidopsis thaliana*." *Plant Physiol.*, 113, 685-693 (In Press).

Ramonell, K.M., Crispi, M.L., and Musgrave, M.E. "Changes in stomatal density in *Arabidopsis thaliana* (L.) Heynh. grown under low oxygen atmospheres." (Abstract) *ASGSB Bull.*, 10, 17 (1996).

Xiao, Y., Kuang, A., Porterfield, D.M., and Musgrave, M.E. "Substrates for growth of *Brassica rapa* in the PGF and SVET." *ASGSB Bull.*, 10, 40 (1996).

Effect of Microgravity on Bone Development

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-03

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303025

Flight Assignment: NIH-R2 (STS-70, June 1995)

Responsible NASA Center: Ames Research Center

Task Description:

This project will study the expression of the tissue plasminogen activator and collagenase enzymes in fetal and postnatal rats exposed to microgravity during development. The findings of this research will throw light on the importance and role of gravity in developing bone.

In FY97, we completed the immunohistochemical analyses of the calvariae from the flight experiments and ground controls, written a manuscript of the work and submitted it to *Calcified Tissue International*. We are awaiting their decision on the review. As well, our work was presented at the 1996 ASGSB meeting. In addition, we have learnt to perform *in situ* hybridization of bone. No changes due to microgravity were observed in the expression of collagenase or tissue plasminogen activator in the calvarial samples, but this may be because exposure to microgravity was during fetal life and these enzymes are maximally expressed 14 days postnatally. This may mean that the recovery period after landing at gestational day 20 (G20) may have abolished any effect of microgravity. Since proposing this work, it has become apparent that collagenase is highly expressed during fetal life in long bones. Thus, we plan to perform *in situ* hybridization of tibiae from G20 pups if we can obtain these from other investigators. We had previously discussed this with Dr. Sue Bodine.

This research will yield new understanding of normal development of bone. It will also aid in our understanding of loss of bone in osteoporosis and osteopenia due to a decrease in loadbearing or immobilization. The information gained may help in the therapeutic intervention of bone diseases on Earth such as osteoporosis.

FY97 Publications, Presentations, and Other Accomplishments:

Fiacco, G.J., Davis, B.A., Sipe, B.D, Jeffrey, J.J., and Partridge, N.C. "The effects of spaceflight on developing rat calvariae: Collagenase and tissue plasminogen activator production." *ASGSB Bull.*, 10, 47 (1996).

Koe, R., Clohisy, J.C., Tyson, D.R., Pulumati, M.R., Cook, T.F., and Partridge, N.C. "Parathyroid hormone versus phorbol ester stimulation of activator protein-1 gene family members in rat osteoblastic cells." *Calcif. Tiss. Int.*, 61, 52-58 (1997).

Partridge, N.C., and Winchester, S.K. "Osteoblast proteinases" in "Principles of Bone Biology." Edited by: J.P. Bilizekian, L.G. Raisz and G.A. Rodan Academic Press, San Diego, pp 207-216 (1996).

Pearman, A.T., Chou, W.-Y., Bergman, K.D., Pulumati, M., and Partridge, N.C. "Parathyroid hormone induces c-fos promoter activity in osteoblastic cells through phosphorylated CREB binding to the major CRE." *J. Biol. Chem.*, 271, 25715-25721 (1996).

Flight-Induced Changes in Immune Defenses

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 105-50-01	Solicitation: 96-OLMSA-01
Initial Funding Date: not available	Expiration: not available
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$150,000	

Flight Information:

Experiment ID: 9601210
Flight Assignment: TBD
Responsible NASA Center: Johnson Space Center

Task Description:

Space flight may affect the delicate host-parasite relationship, thus increasing susceptibility to infectious disease. Changes in the human immune response during space flight suggest that the ability to meet infectious challenges may be attenuated. We shall test the following hypothesis: essential functions of neutrophils, monocytes, and natural killer (NK) cells will be altered during space flight. The constraints inherent in space flight (e.g., few subjects) mandate the use of ground-based models to supplement flight investigations. These models will include a closed population in closed chamber studies. These studies will evaluate quantitative and functional data from three important components of the immune response: neutrophils, monocytes and natural killer cells. Our objectives are to determine the effect of space flight on (1) neutrophil and monocyte functions such as chemotaxis, adherence, phagocytosis, and degranulation, and (2) natural-killer cell and lymphokine-activated killer cell cytotoxicity against targeted cells, and cytokine production. Results from these studies will provide essential data complementing other ongoing space immunology investigations. Realization of our specific aims will increase our understanding of the host-parasite relationship and the risk of infectious disease during space flight.

NK-cell cytotoxic activity and phenotypic expression were determined in peripheral blood mononuclear cells (PBMC) isolated from 11 U.S. astronauts before and after 9-10 days of space flight aboard the Space Shuttle. Specimens were collected 10 and 3 days before launch, within three hours after landing, and 3 days after landing. All PBMC preparations were cryopreserved and analyzed simultaneously in a 4-hour cytotoxicity ⁵¹Cr-release assay using NK-sensitive K562 target cells. Cytotoxicity was assessed in terms of both absolute ⁵¹Cr release and ⁵¹Cr release per 1000 lymphocytes. Compared to preflight values, NK-cell cytotoxicity (corrected for lymphopenia observed on landing day) significantly decreased at landing ($P < 0.0001$). The reduced NK-cell cytotoxicity at landing began an apparent recovery to preflight values within three days after landing. Expression of major lymphocyte surface markers, determined by flow cytometric analysis, revealed no consistent phenotypic changes in NK cells after 10-days of space flight.

Recruitment and sequestration of neutrophils and mononuclear leukocytes are fundamental responses to infection and injury. Altered leukocyte subpopulations, decreased lymphocyte proliferation and decreased cytokine production have been observed following space flight. The mechanisms responsible for these abnormalities are not well defined, and it has been hypothesized that neuroendocrine-mediated factors may play a role in the altered immune response. This study investigated the effects of space flight on leukocyte distribution, stress hormones and immunoglobulin levels, and neutrophil function from US space shuttle astronauts prior to launch (L-10 and 2 days) and after landing (R+0 and R+3 days). At landing, a 2-fold increase in peripheral blood neutrophils was observed primarily due to increased epinephrine and norepinephrine levels. Lymphocytes were slightly decreased in number, and the results were variable for monocytes. After landing, no changes in immunoglobulin classes (IgA, IgG, IgM, IgG, or IgE) were observed, cortisol was slightly decreased, and ACTH was slightly elevated. The neutrophil chemotactic results showed a log decrease in the optimal dose response to fMLP at R+0 or R+3. This suggested an alteration in the ability of neutrophils to respond to fMLP and not in cell number. Neutrophil adhesion to TNF alpha-stimulated human umbilical vein endothelial cells was increased at L-3 and R+0. The expression of MAC-1 (CD11b) and L-selectin (CD62L) was significantly decreased and increased at R+0, respectively. These results indicate that acute response mediators (epinephrine and cortisol) may be partially responsible for alterations in leukocyte subpopulations, differential expression of adhesion molecules, and functional alterations observed after space flight.

Our major goal is to determine if space flight increases the risk of infectious diseases among crew members. Our concern is heightened during long duration missions because changes in the human immune response can have devastating effects upon the health and productivity of individuals. Increased or inappropriate responses leads to numerous hypersensitivity and autoimmune disorders. Decreased capabilities to respond to microbial invaders and tumor cells can lead to increased risk of infectious disease and tumors, respectively. Changes in the immune system have been observed following space flight, and the cause of these changes are not certain. They may result from the psychological and physical stresses associated with space flight. Living and working in microgravity results in some well-known physiological effects and may contribute to the changes observed in the immune response. The major objective of this work is to understand the effects of space flight upon the human immune response. This information is vital to our mission of keeping the crew members healthy. However, these studies may also help to understand immune function on Earth. Microgravity is a perturbation that cannot be duplicated on Earth, and studies of the immune system in space under this unique perturbation may prove to be a valuable tool in understanding the immune response on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Stowe, R.P., Mehta, S.K., Kaur, I., Jones, M.L., Feedback, D.L., Barrett, A.D.T., and Pierson, D.L. "Effect of space flight on neutrophil function." (Poster) 12th Man in Space Symposium, The Future of Humans in Space, Washington, DC (June 8 - 13, 1997).

Gravity-Induced Changes in Gene Expression in Arabidopsis

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Funding:

UPN/Project Identification: 106-50-01 Solicitation: 96-OLMSA-01
Initial Funding Date: 1997 Expiration: 1999
Students Funded Under Research: 2 Post-Doctoral Associates: 0
FY 1997 Funding: \$170,000

Flight Information:

Experiment ID: 9601330
Flight Assignment: BRIC-11 (STS-93 [Target])
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: BRIC

Task Description:

The growth of plants in space is essential for long-term presence of humans in space. Plants, in addition to producing food, can replenish oxygen and remove carbon dioxide and other nitrogenous wastes. Hence, plants play a critical role in developing a self-sustained regenerative life support system in space. Plants on Earth are constantly influenced by gravity, which controls many aspects of their growth and development. The effect of reduced gravity on plants is poorly understood. Hence, it is crucial to elucidate the mechanisms by which gravity affects and controls different aspects of growth and development. Most studies aimed at investigating the mechanisms involved in gravity signal perception and transduction have been conducted on Earth using clinostats or by changing the position of plants with respect to the gravity vector. Such studies have greatly contributed to our understanding of gravity signal perception and transduction. However, these approaches do not eliminate the gravity signal to study the effects of gravity on plants at the cellular and molecular level. The best way to determine the gravity effects is to compare the chosen phenotypic, biochemical or molecular parameters between the plants that are grown in microgravity and terrestrial gravity. Identification of genes involved in gravity signal perception and transduction is contingent upon conducting studies at the molecular level.

The goal of this study is to investigate gravity-regulated gene expression in *Arabidopsis* using Earth- and space-grown seedlings in a Biological Research In a Canister (BRIC) experiment. Specific objectives to meet this goal include:

- (1) To study changes in translatable mRNAs due to gravity. This will be accomplished by isolating mRNA from Earth- and space-grown seedlings and translating the mRNA *in vitro* translation system.
- (2) To isolate genes that are regulated by gravity. Libraries made from mRNA isolated from seedlings grown on Earth and in space flight will be screened by differential plaque filter hybridization.

(3) To analyze the expression of genes, which are known to play a key role in calcium mediated signal transduction pathway, in Earth- and space-grown seedlings. Currently, nothing is known about the effects of gravity on gene expression although there is indirect evidence to indicate gravity regulated gene expression.

The proposed studies represent a first step toward understanding the effects of gravity on gene regulation. *Arabidopsis* will be used as a model system because of a number of advantages that it offers for molecular genetic studies: this plant has been grown in space in past flights. A number of mutants that show altered gravitropic responses are available. Furthermore, several genes involved in calcium signal transduction pathway have been cloned. This information will be used in quantifying the expression of specific genes by polymerase chain reaction in Earth- and space-grown seedlings. Investigations on gravity-induced changes in the gene expression and isolation of gravity-regulated genes are critical to understand the mechanisms by which gravity influences various aspects of plant growth and development. In addition, the knowledge derived from such studies should help in successfully growing plants under microgravity conditions.

We have initiated a BRIC experiment to investigate the effects of gravity at the molecular level using *Arabidopsis*. In preparation for a space flight experiment, a series of ground-based studies were conducted. Results from these studies indicate that: 1) up to 20,000 seeds can be germinated on a 100 mm diameter petri plate, 2) nylon membrane is the best surface for recovery of plant material after freezing, 3) depending on the stage of the seedlings at the time of freezing, 20 to 40 g of tissue can be obtained from petri plates that fit in a single canister, and 4) tissue from one canister yields adequate amounts of RNA to perform differential display to isolate gravity-regulated genes. Our results indicate that the proposed BRIC experiment is feasible and can provide valuable information on the possible effects of microgravity on gene regulation.

In order to grow plants in space it is essential to understand the effects of microgravity on growth and development, especially at the molecular and cell biological level. Our studies will determine the effects of microgravity on gene expression and should provide new insights in to how plants sense and respond to gravity signal. The knowledge obtained from these studies will be useful for the development of bioregenerative life support systems to support crews in the space environment. Furthermore, these studies could also lead to advances in plant biotechnology and medicine.

FY97 Publications, Presentations, and Other Accomplishments:

Reddy, A.S.N., Kao, Y-L., Mykles, D., Sadeh, W., and Wheeler, R. "Studies for a BRIC experiment to investigate gravity-induced changes in gene expression." 26th Intl. Conf. on Environmental Systems, Paper No: 961394, pp. 1-6.

Microgravity and Placental Development

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 5-01131

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303012

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Animal Enclosure Module

Task Description:

This experiment will use pregnant rats to determine the effect of microgravity in development of the rat placenta. Ten pregnant rats will be aboard the space shuttle during its 11-day mission. Upon return to Earth, the rat uteruses and placentas will be examined. Morphological, biochemical, and endocrine variables of these tissues will be analyzed to determine whether the cells involved retained their structure and are operating correctly. These studies could identify factors that regulate pregnancy and provide insights into the role that gravity plays in pregnancy on Earth.

RNA was recovered from tissue samples, separated by electrophoresis, and blotted to membrane for Northern analysis. cDNA probes for glucose transmitters 1 and 3 were unable to detect message for these molecules making it impossible to evaluate the effect of space flight. Technical difficulties have slowed attempts to develop more sensitive RNA probes that may be able to detect glucose transporter mRNA in these samples. Project funding ended April 30, 1997; however, we continue to pursue the this objective using other funding sources.

Infertility is a health problem which may lead to significant psychological and economic stress for many couples. This malady may result from processes associated with gamete fertilization, embryo implantation, or placental insufficiency. This project was designed to study the latter of these possibilities by examining the role of gravity in placental growth and development. It was hypothesized that the correct vectoral movement of cells during embryo implantation and placental development is dependent upon gravitational forces. In addition, hemodynamic changes associated with microgravity were postulated to adversely influence placental development and function. There is a need to know the effect of space flight on reproductive processes at this time to

determine if studies of mammalian biology which would require stable animal colonies aboard an orbiting laboratory can be planned. Also, a better understanding of reproductive processes will facilitate management of human proliferation in face of a finite supply of resources on Earth.

Early Development of Fern Gametophytes in Microgravity

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 95-OLMSA-02

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 120,000

Flight Information:

Experiment ID: 95020368

Flight Assignment: STL-B-02 (STS-93, 1997) and BRIC-12 (TBD)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC, STL-B

Task Description:

The overall objective of this Small Payloads research project is to determine the effects of microgravity on nuclear migration, early polar development and gene expression in gametophytes of the fern *Ceratopteris*. The gametophyte begins as a single spore cell. Nuclear migration occurs during spore germination, and the downward direction of its movement is specified by gravity several hours before the migration begins. The lower position to which the nucleus migrates sets the polarity of the first cell division, which is asymmetric and places the smaller cell below the larger one. This, in turn, dictates further development, for the smaller cell gives rise to the rhizoid and the larger one gives rise to the protonemal initial, the progenitor of the prothallus. This proposal will investigate whether the vector force of gravity is needed to direct the first polar movement of the nucleus and the subsequent polarity of the first cell division, whether this polarity is needed for the normal development of rhizoid and prothallus structures, and whether germinating spores in microgravity exhibit differential gene expression compared to 1-G controls. Nuclear migration and post-germination development will be video recorded through a microscope in a middeck locker using the Space Tissue Loss (STL-B) equipment described in the research announcement. Spores will be frozen in space at various times and their mRNA population at the time they were frozen will be compared to that of 1-G controls germinated on Earth and frozen at the same time, using sensitive differential gene expression screening methods. Expected results should satisfy the Space Biology Program emphases in cell, developmental, and plant biology.

Progress during fiscal year '97 was primarily toward the achievement of two goals: (1) Optimize specific parameters and conditions by which to conduct experiments aboard a small payloads flight of the space shuttle, and (2) Document differential gene expression during the period in which fern spores respond to gravity.

For Goal 1, the optimal parameters and conditions were found to be as follows:

a) illumination - conditions were found to be white fluorescent light with an intensity of 2650 lumens and

wavelength spectral distribution of 450 nm - 700 nm. The light source for illumination to acquire the video must be scattered and not unidirectional.

- b) temperature - Temperature control is necessary within the range of 29 ± 2 °C.
- c) sample selection - Spore batches differing in harvest time and conditions were tested, and one was chosen that has a "window" of gravisensing that accommodates the duration of the space flight, is highly synchronous, and is available in large quantities for further genetic studies.
- d) sample preparation - Experiments were conducted to identify the optimal agarose concentration in which to embed the spores inside the optical biochamber wells of the STL- B. Nuclear migration and rhizoid emergence were observed in all concentrations tested; however, it was found that 0.5% was the most concentrated solution in which growth of rhizoids was not retarded.

For Goal 2, we evaluated differential gene expression during the period in which the spores respond to 1-G (called the "gravity window") using the technique of Differential Display RT-PCR. This technique yields candidate cDNAs that *could* represent mRNAs that are differentially expressed during the gravity window, but these must be tested by Northern analysis to be confident that they are *actually* differentially expressed. Because the candidate cDNAs are typically less than 300 bp, a smaller than ideal size to use as a probe for Northern, we tried to obtain more full-length versions of these. To do this we constructed and screened a cDNA library based on mRNAs isolated from gametophytes, and we used 5'RACE, a standard methodology used to obtain additional 5' sequence of less-than-full-length cDNAs. Finally, to evaluate the quality of the mRNA we used for library construction, RACE and Northern analyses, we carried out *in vitro* translation and Iso Electric Focusing. Results obtained are summarized below under the heading of the technique used (as highlighted in bold).

Differential Display RT-PCR - Over 20 candidate cDNAs have been identified to be differentially expressed using only 1 primer combination of the possible 96 combinations that would encompass the entire genome. Candidate cDNAs were excised from the gel, eluted, and reamplified. Seven of these candidates were subcloned.

Northern Analysis was performed using 7 of the partial cDNAs as probes. They all recognized mRNAs that were in the range of 1.5 to 2.5 kb and were upregulated during the gravity window. However these results will be more rigorously tested using longer versions of the cDNAs as probes.

cDNA library construction and screening - A cDNA library was constructed from gametophytes of *Ceratopteris*. This library was amplified to obtain a final titer of approximately 6.9×10^{11} pfu/ug. Initial sequence analysis of clone selected at random from the library shows high homology to various *Arabidopsis* proteins. This library is currently being screened with probes obtained from DDRT-PCR.

5' RACE - Two of the cDNAs subcloned from those selected by DDRT-PCR (see above) were sequenced, and from these sequences primers were designed to carry out 5' RACE on these cDNAs. From the longer of these two cDNAs (456 bp), an additional 700 bp of 5' sequence was obtained for a total of 1156 bp of sequence. To verify that the two cDNAs (456 bp and 700 bp) were indeed from the same sequence, Northern analysis was performed and observed for hybridization to the same location and identical size gene. Both cDNAs showed identical hybridization patterns and size, confirming they are from the same cDNA. They recognize two mRNAs, at near 5.0 kb and 2.5 kb, which could represent alternate splicing products of the same gene or mRNAs of two closely related genes that share some portion of their sequence in common. The mRNAs recognized show a dramatic upregulation at approximately 42 h after germination, when 85% of the spores have responded to gravity. They disappear at 66 hours after germination, at which time 89% have responded to gravity. For the second cDNA used for 5'RACE (179 bp), an additional 1000 bp of sequence was obtained. This is currently being cloned and sequenced.

***In vitro* translation and Iso Electric Focusing** - mRNA was extracted from spores which show 90% gravireponsive behavior. This mRNA was used for *in vitro* translation and Isoelectric focusing experiments to identify the proteins that are present at this time. Preliminary results showed approximately 50 - 100 proteins

This project uses plants as subjects and does not seek to understand a human disease or develop new therapeutics for alleviating any disease on Earth. However, it does aim to generate a new understanding of basic biological processes, namely the fundamental cellular mechanisms whereby cells sense and respond to gravity. The specific emphasis of the project is on trying to identify genes that must be expressed in order for single fern cells to use gravity to fix the polarity of their nuclear migration and subsequent development. Expectations are that the study of these genes and the proteins they encode will help identify fundamental components of the gravity sensing/responding machinery in fern cells, and that some of the insights derived from this study will be applicable to at least some other cell types. The shuttle flight stage of this project will allow an evaluation of whether genes that are expressed during the gravity-responsive period of fern spore germination on Earth are also expressed in microgravity, and whether the virtual absence of a gravity signal inhibits the nuclear migration event or merely randomizes the direction of migration. These results, in turn, will reveal whether the force of gravity influences patterns of gene expression important for fern cell development. Overall, this project will contribute important new data relevant to the intensely debated question of what aspects of cell function are altered in microgravity, a question whose answer has major implications for the future of humans in space.

FY97 Publications, Presentations, and Other Accomplishments:

Chatterjee, A. and Roux, S.J. "Differential gene expression in germinating spores of *Ceratopteris richardii* during the period of responsiveness to gravity." *ASGSB Bull.*, 10, 18 (1996).

Edwards, E.S. and Roux, S.J. "The influence of gravity and light on developmental polarity of single cells of *Ceratopteris richardii* gametophytes." *Biol. Bull.*, 192, 139-140 (1997).

Roux, S.J. "Influence of gravity and light on developmental polarity of single gametophytic cells of *Ceratopteris richardii*." International Meeting on Fern Development and Evolution, Purdue University (July, 1997).

Differentiation and Tropisms in Space-grown Moss (Ceratodon)

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: not available

Joint Agency Participation: NSAU

Flight Information:

Experiment ID: 9600005

Flight Assignment: CUE (STS-87, November 1997)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC-LED, PDFU

Task Description:

Protonemata of the moss *Ceratodon* are tip-growing cells that grow up in the dark. This cell type is unique compared to cells in almost any other organism since the growth of the plant cell itself is completely oriented by gravity. Thus, both the processes of gravity sensing and the gravity response occur in the same cell. Gravity sensing appears to rely upon amyloplasts (starch-filled plastids) that sediment. This sedimentation occurs in specific zones and plastid zonation is very complex with respect to plastid morphology, distribution, and gravity. Microtubules may function both in producing gravitropic curvature as well as in controlling plastid sedimentation. Since gravity plays crucial roles in the growth and organization of this highly specialized cell, it is important to determine whether this cell differentiates normally in microgravity. Protonemata are excellent subjects for space flight experiments since they are readily cultured in sealed containers in the dark. Thus they can be grown in a BRIC and fixed in position in space.

Ceratodon protonemata are also an excellent system for the study of gravity on the organization since differentiation is influenced by light. Thus, it is possible to study the effects of microgravity on several different cell types. Since these cells are phototropic (grow towards unilateral light) as well as gravitropic, both ground-based studies and space flight offer an opportunity to resolve whether both phototropism and gravitropism occur simultaneously.

In many plants, starch content is influenced by gravitational stress and by microgravity. Since *Ceratodon* protonemata contain different types of amyloplasts (statolith and storage), it will also be meaningful to test the effects of flight on starch content and metabolism.

A key component of this mission is the involvement of Ukrainians. All Ukrainian scientists collaborating on this moss experiment have been working for many years in both ground- and space-based studies in moss gravitational biology.

In the last year, the culture and experiment conditions for the two moss species *Ceratodon purpureus* (US part of the experiment) and *Pottia intermedia* (Ukrainian part) have been optimized. The light quality, light intensity and the experiment treatments for the space flight were defined and finalized.

Another major focus has been to help design and test purpose-built flight hardware. One component is a BRIC canister that was modified to provide lateral red-light illumination of the moss cells using light emitting diodes (BRIC-LED). Another component is a special petri dish fixation unit (PDFU) that makes it possible to chemically fix biological samples in place (inside the dish) while growing in microgravity. Each BRIC-LED contains 6 dishes and has external controls that contain switches to initiate fixation and to control the timing of illumination. These two integrated pieces of hardware will undoubtedly be useful for many other space experiments. Equipment verification and modification required four separate extended runs (SVTs and PVTs). Modifications also involved minimizing heat generation and overheating of the moss samples by mounting a fan into the flight middeck locker.

Ground-based reference experiments were performed to determine the relative importance of gravitropism and phototropism. It was found that at higher light intensities, phototropism completely overrides gravitropism. This was determined by turning cultures at various angles with respect to gravity and with respect to a simultaneous illumination with unidirectional light so that the light and gravity vectors acted either in the same or in different directions. Unilateral red light for 24h at intensities $\geq 140 \text{ nmol m}^{-2} \text{ s}^{-1}$ caused the majority of protonemata to be oriented directly towards the light. Similarly, protonemata grew directly towards the light regardless of light position with respect to gravity indicating that at these light intensities all growth is oriented strictly by phototropism, not gravitropism. However, at very low light intensities, no phototropism seems to occur and mean protonemal tip angle is above the horizontal; this indicates that low intensity red light permits some gravitropism but also modulates the response so that it is not strictly upright. Protonemata of an aphototropic mutant of *Ceratodon*, *ptr1*, which lacks a functional phytochrome chromophore, exhibit gravitropism regardless of red light intensity. These data indicate that red light acts via phytochrome to modulate gravitropism at low intensities and to completely suppress gravitropism at intensities $\geq 140 \text{ nmol}$. The effects of both intensities of red light will be tested in microgravity.

The focus of upcoming ground reference experiments will be to compare the distribution of plastids in stationary protonemata (upright and inverted) with cells that have been rotated on a clinostat. Although plastid sedimentation occurs along the length of dark-grown cells, only some plastids fall and they do not fall all the way to the bottom of the cells. Moreover, microtubules appear to be load-bearing for many of these plastids. The distribution of plastids in microgravity may indicate whether these microtubules are normally under tension. These data are relevant to an understanding of the role of the cytoskeleton in maintaining cell organization against the force of gravity in eukaryotic cells.

Moss protonemata grow solely by tip growth, that is the most apical cell of the filament extends only at its tip. Dark-grown protonemata of mosses such as *Ceratodon*, *Physcomitrella*, *Funaria*, and *Pottia* grow upward in the dark and are thus negatively gravitropic. Gravitropic, tip-growing cells of plants are unique compared to cells in almost any other organism, since the growth of the plant cell itself is completely oriented to gravity. Protonemata of the moss genus *Ceratodon* are among the most vigorous and rapid gravitropic tip-growing cells known. Gravity sensing seems to take place continuously in *Ceratodon* protonemata. The apical tip cell contains amyloplasts that sediment in a specific zone just behind the apex and several lines of experimental evidence suggest that this sedimentation functions in gravity sensing. These cells offer many experimental advantages for studying the effects of gravity since they: (1) represent one of the few gravitropic tip-growing systems known, (2) contain both the processes of gravity sensing and the gravity response in the same cell, (3) are also phototropic and light influences gravitropism, and (4) are readily cultured in agar in sealed containers in the dark. Although space flight experiments have been conducted with moss protonemata (*Funaria hygrometrica* on board the Russian orbital space station Salyut 6, 1979), none involved gravitropic cells.

Ceratodon protonemata are an excellent system in which to study the interactions of phototropism with gravitropism and these experiment features will reveal a new understanding of these basic biological processes.

FY97 Publications, Presentations, and Other Accomplishments:

Kern, V.K. and Sack, F.D. "Relationship between gravitropism and phototropism in protonemata of the moss *Ceratodon*." (Abstract) *Plant Physiol.*, 114: (Suppl), 623 (1997).

Sack, F.D. "Plastids and gravitropic sensing." *Planta*, 203, S63-S68 (1997).

Wagner, T.A., Cove, D.J., and Sack, F.D. "A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon purpureus*." *Planta*, 202, 149-54 (1997).

Wagner, T.A., Cove, D.J., and Sack, F.D. "A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon*." (Abstract) *Plant Physiol.*, 114: (Suppl), 618 (1997).

Walker, L.M. and Sack, F.D. "Stereological analysis of gravitropism in protonemata of the moss *Ceratodon*." *Int. J. Plant Sci.*, 158, 24-31 (1997).

Microgravity Effects during Fertilization, Cell Division, Development, and Calcium Metabolism in Sea Urchins

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: A144 BF78 A48 9700
Initial Funding Date: not available
Students Funded Under Research: 4
FY 1997 Funding: \$70,000

Solicitation: 95-OLMSA-01
Expiration: not available
Post-Doctoral Associates: 1

Flight Information:

Experiment ID: 9501376
Flight Assignment: ARF-1 (STS-77, May 1996)
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: ARF

Task Description:

Gravity has been shown to affect bone calcium, and it may well influence processes during fertilization, cell division, development, and embryogenesis. This project explores the role of microgravity during fertilization, early development, cytoskeletal organization, and skeletal calcium deposition in a model developmental system: the sea urchin. We have helped the Canadian Space Agency (CSA) develop, test, and fly the aquatic research facility (ARF) system, and have documented the events in living eggs during fertilization, including the physical incorporation of the sperm into the egg and the union of the maternal and paternal genomes by light and electron microscopy on ground and in space. The organization of the cytoskeleton responsible in eggs and embryos for the movements during fertilization, cell division, and embryogenesis was documented with immunofluorescence (epifluorescence and confocal) microscopy to localize microtubules, microfilaments, and other cytoskeletal proteins as well as with high-resolution, high-voltage electron microscopy (HVSEM).

Sea urchins were flown in the ARF system aboard the space shuttle Endeavor during the STS-77 mission in May 1996. In-flight fertilization of six cultures was successfully accomplished by astronaut Mario Runco using the newly designed Fertilization Syringe Unit (FSU). Six other unfertilized egg cultures were fertilized in a 1-G centrifuge in space to mimic gravity conditions on Earth and to serve as control to the cultures grown in 0-G. Post-flight analysis revealed that secretion of cortical granules was decreased and incomplete in a large number of eggs fertilized in microgravity compared to eggs fertilized on ground. Furthermore, microvilli as the surface of eggs fertilized in microgravity were shorter which is interpreted as inhibition of microfilament assembly.

The methods developed for this project have been applied to one-cell, two-cell, eight-cell, and sixteen-cell embryos, as well as to blastulae, gastrulae, and pluteus stages grown in the chambers specifically designed for space shuttle experimentation. We have used fluorescently labeled anti-tubulin antibodies to detect

microtubules, fluorescently labeled anti-centrosomal antibodies to detect the microtubule-organizing centers, and the fluorescent compound Hoechst 22358 to visualize chromosomes. We have also performed high resolution electron microscopy to detail the molecular components of cytoskeletal structures at 0-G and 1-G. Post-flight analysis of the centriole-centrosome complex indicates that it is affected in a small number of dividing cells. This finding leads to the conclusion that the centrosome-centriole complex may be gravity-sensitive.

To investigate the effects of microgravity on calcium metabolism and the cytoskeleton during fertilization, cell division, cell differentiation, and early development, sea urchins were fertilized in space to follow the sequence of microfilament and microtubule organization under microgravity conditions. Eggs and embryos were maintained in Standard Container Assemblies (SCAs) with identical sets prepared for culture in microgravity, and at 1-G on the space shuttle Endeavor during the STS-77 mission, as well as for ground observation at the Kennedy Space Center. Concentrated fixation fluid was injected into the SCAs at preselected specific time points at a final concentration of 0.5% glutaraldehyde and 4 μ m taxol to preserve microtubules, centrosomes, centrioles, microfilaments, mitochondria, membranes, and Golgi complexes. For post flight analysis of cell biology events during development, light, (immuno)fluorescence, scanning, and transmission electron microscopy were employed. Preliminary analysis of samples flown during the STS-77 mission indicates that fertilization took place in microgravity but the signal transduction at the egg plasma membrane was altered which resulted in a decrease in cortical granule exocytosis and microfilament formation. Cell division and development was slower in microgravity as compared to 1-G controls in space and on ground which may be an indication for cellular adaptation to microgravity. It was also noted that a small percentage of cells divided abnormally in microgravity which may indicate that centriole separation and orientation was affected. The methods developed for this project have been applied to one-cell, two-cell, eight-cell, and sixteen-cell embryos, as well as to blastulae, gastrulae, and pluteus stages grown in the chambers specifically designed for space shuttle experimentation. We have used fluorescently labeled anti-tubulin antibodies to detect microtubules, fluorescently labeled anti-centrosomal antibodies to detect the microtubule-organizing centers, and the fluorescent compound Hoechst 22358 to visualize chromosomes. We have also performed high resolution electron microscopy.

The experiments conducted in this project are investigating basic cellular functions. By determining the sites of dysfunction, strategies may be developed that identify target sites for pharmaceuticals that could correct affected areas. Investigating the cytoskeletal system including centrosomes will lead to understanding underlying molecular mechanisms for disease. Centrosomes, and centrioles in particular, are poorly investigated although they play key roles in processes where microtubule function is required. This project will contribute to understanding the cytoskeleton in biological processes such as fertilization, cell division, cell differentiation, embryogenesis, nerve cell function, muscular function, and environmental effects on bone structure. Astronauts traveling in space are subjected to calcium loss as much as human who are aging or are unable to use their muscles. Research on the cytoskeleton and skeletal formation will benefit processes on Earth and in space. This research will provide data for the design of pharmaceuticals that could directly benefit the common man affected by diseases such as osteoporosis, cancer, and neurological diseases including Alzheimer's.

FY97 Publications, Presentations, and Other Accomplishments:

Schatten, H. "Culture of sea urchin embryos in the new aquatic research facility (ARF) and first results from its space voyage on STS-77." American Society for Gravitation and Space Biology Annual Meeting, Charlotte, NC (October 23 - 26, 1996).

Schatten, H. "Cytoskeletal organization and function during the first reproductive cell cycles in sea urchins and mice." UMKC Kansas City (November 26, 1996).

Schatten, H. "Cytoskeletal organization during fertilization, cell division, and development in microgravity: Results from a space shuttle flight (STS-77) on the Shuttle Endeavor." MIKMAS - Central States Microscopy Society Meeting (October 30, 1996).

Schatten, H. "Experimental modification of centrosome shape with chloral hydrate supports the string model of the centrosome." Memorial Symposium in Honor of Daniel Mazia, Pacific Grove, CA (December 4 - 6, 1996).

Schatten, H. "Sea urchin culture in space: Results from experimentation on STS-77." UMC Department of Veterinary Pathobiology (February 11, 1997).

Schatten, H. "The centrosome-centriole complex during sea urchin development. Light and electron microscopy analysis." American Society for Cell Biology (December 7 - 11, 1996).

Schatten, H. and Chakrabarti, A. "Culture of sea urchin embryos in the new aquatic research facility (ARF) and first results from its maiden space voyage on STS-77." (Abstract) ASGSB Bull., 10(1) (1996).

Schatten, H., Chakrabarti A., and Hedrick J. "Cytoskeletal organization is altered by microgravity when sea urchin embryos are cultured under space flight conditions." UMC Molecular Biology Program (March, 1997).

Schatten, H., Chakrabarti A., Hedrick J., Bigus L., and Stogsdill P. "Microgravity disrupts cytoskeletal organization and causes similar effects as observed in cells during aging." UMC Molecular Biology Program (March, 1997).

Schatten, H. (Session Chair) "Cytoskeletal organization during fertilization, cell division, and development in microgravity: Results from a space flight (STS-77 on the Shuttle Endeavor) ." MIKMAS - Central States Microscopy Society Joint Fall Meeting, University of Missouri-Columbia (October 18, 1996).

Brain-Pituitary Axis Development in the CEBAS Minimodule

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Funding:

UPN/Project Identification: not available

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 10

Post-Doctoral Associates: 1

FY 1997 Funding: \$128,000

Flight Information:

Experiment ID: 9501137

Flight Assignment: CEBAS (STS-89, January 1998)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: CEBAS Minimodule

Task Description:

The CEBAS minimodule system is a man-made aquatic ecological system that incorporates animals, plants, snails, and microorganisms. It has been proposed that the CEBAS will lead to a multigeneration experimental facility for utilization in a space station as well as for the development of an aquatic CELSS to produce animal and plant biomass for human nutrition. In this context, research on the reproductive biology of the organisms within the system should receive the highest priority. Thus, the goals of our proposal are to provide information on space-flight-induced changes in the brain-pituitary axis and in the organs that receive information from the environment in the vertebrate selected for the CEBAS Minimodule program, the freshwater teleost *Xiphophorus helleri* (the swordtail). We will study the development of the brain-pituitary axis in embryos, neonates, immature and mature swordtails using histology, cytology, immunohistochemistry, morphometry, and *in situ* histochemistry to evaluate the synthesis, storage, and release of neurotransmitters, neuroregulatory peptides, neurohormones, and pituitary hormones as well as the structure of the organs and cells that produce, store, or are the target organs for these substances. Similar methods will be used to study the pineal organ and the olfactory system.

This research represents a logical sequence of studies, from laboratory to space flight, that will provide seminal, essential information of the effect of space travel conditions on the development and functioning of the neuroendocrine system regulating the reproductive system.

We have participated in two PVTs, one in July and one in August, which correspond to the Small Payload Mission in January and the Neurolab Mission in April, respectively. We have processed and begun the evaluations of the tissues derived from these two PVTs, according to our proposed protocols. In addition, we are tracking the growth and development of neonates from these tests that were returned to our laboratories in Brooklyn College.

The implications of studying a vertebrate in space flight with a reproductive system similar to mammals is patent. Additionally, knowledge of the reproductive system of fish may serve to introduce the consideration of aquaculture as a means of generating animals protein into life support systems.

FY97 Publications, Presentations, and Other Accomplishments:

Cepriano, L. and Schreibman, M.P. "Fish for launch." Presented at U.S. National Park Service Brunch on the Bay Series (July, 1997).

Flynn, K.M., Schreibman, M.P., and Magliulo-Cepriano, L. "Developmental changes in NMDA receptor expression in the platyfish brain." *Brain Res.* (In Press).

Flynn, K.M., Yablonsky-Alter, E., Banerjee, S.P., and Schreibman, M.P. "NMDA receptor dynamics in GnRH Producing cells: Changes with sexual maturity." *Soc. Integrative. Comp. Biol.* (December, 1996).

Yousha, E.J., Bluem, V., and Schreibman, M.P. "Identification and sequencing of a chicken II GnRH PCR product from genomic *Xiphophorus lancestilia* DNA." *Soc. Integrative. Comp. Biol.* (December, 1996).

Effects of Microgravity on Microbial Physiology

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Funding:

UPN/Project Identification: 106-50-10 (E562)

Solicitation: 93-OLMSA-07

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$265,000

Flight Information:

Experiment ID: 9307562

Flight Assignment: MPFE-01 (TBD)

Responsible NASA Center: Johnson Space Center

Flight Hardware Required: AMS (Automated Microbiology System)

Task Description:

Microorganisms are capable of quickly adapting to changes in their environment. Metabolic pathways adjust to new environmental conditions to optimize growth and ensure survival. Environmental adaptation is a key tool used in microbial research to investigate the basic physiology of microbial species. Temperature, pH, and growth media constituent manipulation are classic examples of environmental parameter control schemes used to induce observable metabolic changes. While classic induced physiology adaptation has been studied extensively, the effects of gravity on microbial physiology remain essentially unknown. Prior space flight research indicates that exposure to microgravity can lead to increased growth rates and increased resistance to antimicrobial agents. However, the limited data and conflicting results of this body of work demand that a well-controlled, prolific methodology be adapted for microbial space flight research. The hypothesis to be tested here is that microgravity will induce functional changes in microbial physiology. To identify and evaluate these changes, a two-phase project will be conducted to first screen for and then target statistically significant differences in physiological behavior between ground-based and on-orbit microbial cultures. The screening phase of the project will investigate the growth, substrate utilization characteristics, and reaction to antimicrobial agents of twelve microbial strains including bacteria and yeasts. These microbes will be analyzed simultaneously on-orbit and on the ground using an automated microbial analysis technology, the Vitek System manufactured by bioMerieux Vitek, Inc. Microbial growth patterns will be measured in standard Vitek identification and antibiotic susceptibility test cards. A physiological profile consisting of observed growth rates, substrate utilization characteristics, and antimicrobial susceptibilities will be obtained for each microbial strain. Physiological profiles from the terrestrial and on-orbit microbial analyses will be compared to determine statistically significant differences in behavior. In the second phases of the project, Vitek test cards containing unique media formulations and/or antimicrobial agents will be developed to further investigate each of the specific metabolic

differences identified in the screening phase. These test cards will be analyzed in subsequent shuttle flights to elucidate the cellular mechanisms responsible for the observed metabolic anomalies. This study will provide a basic understanding of the effects of microgravity on microbial physiology. In addition, the antimicrobial physiological profile data will aid in the development of effective therapeutic treatment for controlling on-orbit infectious disease. Adaptation of the Vitek instrument for space flight will provide the first ever on-orbit comprehensive microbial identification capability, an invaluable capability for clinical and environmental microbiology on future long-duration space missions.

Progress on the Microbial Physiology Flight Experiment (MPFE) in FY 1997 has been made in the areas of hardware development and ground support analyses. The flight back-up Automated Microbiology System (AMS) unit was completed and awaits functional testing at bioMerieux Vitek and subsequent Certification/Acceptance testing at NASA-JSC. AMS flight unit components were fabricated and prepared for final assembly and check-out.

Two complementary ground support experiments were performed to determine the stability of the prospective microbial test strains while in refrigerated storage. Flight experiment protocol requires that the flight Vitek test cards be inoculated on ground and placed in refrigerated stowage to prevent premature growth of the microorganisms. Standard procedure on ground would entail immediate analysis of the cards in the AMS after inoculation. Since the flight cards cannot be analyzed immediately, there was concern that delayed analysis and refrigerated stowage of the test strains would confound the results of the on-orbit analyses with phenomena unrelated to exposure to microgravity. A long-term and follow-on short-term stability test were developed to evaluate the stability of the test microorganisms while in refrigerated stowage.

The long-term test consisted of analyzing the test microbes in a commercial AMS instrument after 8 and 13 days of refrigerated stowage and comparing test results with Day 0 controls. As expected, there was a great deal of variability in the response of the various strains. While many of the Gram positive and Gram negative strains maintained their characteristic growth pattern in the Vitek identification test cards, their susceptibility to antibiotics changed considerably with a drift toward higher sensitivity over the course of the experiment. Many of the yeast strains and a few of the bacterial strains were found to be stable at least through 8 days of refrigerated stowage.

Those strains that did not remain stable over the course of the long-term stability analysis were included in the short-term stability test. This experiment consisted of an analysis of the various strains on days 2, 4, 6, and 8 after inoculation. These results were again compared to Day 0 controls for determination of stability. Most of the strains were found to be stable with respect to identification growth patterns and susceptibility for some period of refrigerated stowage. A characteristic maximum stowage duration was assigned to each strain to assist in development of on-orbit protocols and prevent acquisition of degraded experimental data.

The MPFE is aimed at identifying gravity-dependent physiological processes by comparing the growth and metabolism of a broad range of microorganisms cultured in space and on the ground. The anticipated results will indicate which microbes and specifically which metabolic pathways are affected by variation in the gravitational environment. These results will benefit the fields of biology, clinical microbiology, and applied microbiology.

From a basic biological research perspective, identification of gravity-dependent physiological processes will add significantly to our overall understanding of how the environment, and gravity in particular, affects the basic processes of life. Bacteria and yeast are some of the simplest forms of life, yet they exhibit physiological pathways that have been conserved through millions of years of evolution. In this way, microbes represent the ideal model for studying the most basic life processes. The simplicity of these organisms allows for targeting of specific bioprocesses without the complications of intercellular and system-level interactions. Gravity represents an under-utilized tool in the array of environmental parameters used to elicit metabolic responses. Gravity is unique in this array in that it is the one environmental constant under which all forms of life evolved. For this reason, it will be especially intriguing to observe how microbes adapt to an environmental parameter which their genetic programming has never encountered. One might suggest that observed microbial adaptive behaviors may be extrapolated to help explain observed adaptive physiological phenomena in humans as well.

Clinical microbiology is a field which may benefit significantly from the MPFE project. Prior work has indicated that microbes exhibit an increase in resistance to antimicrobial agents when grown in microgravity. If this behavior can be confirmed, the flight experiment will provide an excellent opportunity to investigate in detail the mechanisms of antibiotic resistance. The growing prevalence of resistant pathogen strains has made antimicrobial research of critical importance to the health of the public as a whole. Results of the MPFE project will provide a unique set of data for the clinical microbiology community to use in combating infectious disease.

Gravity-dependent bioprocesses are of considerable interest in the field of applied microbiology as well. Biochemists and crystallographers currently take advantage of the minimal gravity on the Shuttle in Earth orbit to produce crystals that would otherwise not be possible. Physicists researching combustion and phase-change phenomena utilize the unique conditions of microgravity to investigate processes that cannot occur here on Earth. What about biological processes? Biological gravitational effects are not so easily anticipated as those for physical phenomena, yet it is these effects which one hopes will make space processing a valuable commodity. This project will constitute the first steps in the process of identifying those microgravity-induced bioprocesses which may be harnessed to produce unique biochemicals and pharmaceuticals in space.

Effect of Spaceflight on Development of Immune Responses

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Funding:

UPN/Project Identification: not available

Solicitation: 93-OLMSA-03

Initial Funding Date: 1994

Expiration: 1996

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9303009

Flight Assignment: NIH-R1 (STS-66, November 1994)

Responsible NASA Center: Ames Research Center

Task Description:

Space flight has been shown to change immune responses, which are those responses of the body that protect people and other animals from infection. These changes in immune responses could be due to the very low gravity found in space, as well as to other factors such as stress. Changes in immune responses could have an impact on the body's ability to resist infection. The current flight study will look at the effects of space flight on immune responses of developing rats.

Task work is completed. A manuscript is in preparation.

This study has been designed to determine the effects of space flight on development of immune responses in offspring of flown pregnant rats. It should provide new information regarding the normal development of the immune response. This information could prove useful in enhancing the understanding of the development of the immune response in humans. Such understanding could provide new information that may be potentially applicable to understanding the mechanism of and treatment human childhood immunological disorders.

FY97 Publications, Presentations, and Other Accomplishments:

Sonnenfeld, G. "Immune responses in space flight." *Int. J. Sports Med.* (In Press).

Role of Thyroxine in Space-Developed Jellyfish

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Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-07

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 120,000

Flight Information:

Experiment ID: 9307296

Flight Assignment: Deselected

Responsible NASA Center: Kennedy Space Center

Task Description:

The metamorphosis process which enables the formation of ephyrae from polyps is influenced by a hormone, jellyfish thyroxine (Jf-T4), which is synthesized following iodine administration. Two groups of polyps in space (but not controls) formed ephyrae without iodine administration (SLS-1). In addition, in space, jellyfish ephyrae from Earth lost most statoliths and swam/pulsed abnormally. Since thyroxine is known to cause demineralization of statoliths and abnormal pulsing on Earth, these findings suggest that Jf-T4 synthesis, utilization, or secretion may be different in space as compared with ground controls.

Our tasks this year were focused on preparing for the SVT for the ARF-2 jellyfish-in-space experiment. Our efforts included growing and testing numerous jellyfish for normality, including the testing of polyps and ephyrae in an SCAs (Sample Container Assemblies) of the ARF (Aquatic Research Facility) provided by the Canadian Space Agency. In addition, methods for the fixation of jellyfish for ultrastructural studies and for the measurement of Jf-T4 were improved.

Our efforts this year were directed toward preparing the jellyfish experiment for an SVT in October and ultimately for their flight in the ARF-2 hardware. For this purpose, 2025 jellyfish were used for 22 tests which were done @ 23.5°C, usually for 11 days to simulate a 10 day flight (plus 1 day). In addition, extensive tests to determine the best fixation method were done and methods for the quantitation of the Jf-thyroxine hormone were explored. In spite of this extensive testing which achieved the readiness of the jellyfish for the SVT, the jellyfish were abruptly "deselected" for the ARF-2 flight as of August, 1997 and further funding was withdrawn.

Ephyrae Development Tests: To determine the best number of medium-sized polyps to use in the SCAs (Standard Container Assembly - 1 aquarium plus 1 fixative block), seven tests were done using between 30 and 50 polyps. We determined that chambers containing 35 polyps gave rise to ephyrae which were as normal as control ephyrae developing in tissue culture flasks. We therefore planned to use 30 polyps in the SCAs

dedicated to studying ephyra development in micro-G to insure that any possible abnormalities found in micro-G organisms were not due to crowding in the chamber.

Demineralization Tests: Five demineralization tests using newly-released ephyrae were done in the SCAs. Whereas 40 ephyrae/chamber were determined to be excessive for survival for 11 days, 30 ephyrae were in good condition in the SCAs after 11 days. Demineralization of statoliths proceeded as anticipated in these tests with ephyrae still having between 64% and 82% of base-line control numbers at the end of the test period. It is important that the ephyrae did not lose all of their statoliths under these 1-G conditions because it was anticipated that statolith loss will be greater under micro-G conditions during flight (based on SLS-1 jellyfish flight experiment results).

If-T4 Hormone Synthesis Tests: To determine the maximum number of polyps which can be maintained in the SCAs and which can strobilate to the 1 segment stage after being induced with KI late in the mission, we did nine tests. After thoroughly cleaning the chambers and rinsing the polyps, we compared numbers of 100, 150, 200, 225, 300, and 350 polyps in the chambers after 11 days. We found that, while 350 polyps lived in the chambers 11 days and most of them formed 1 segment, others did not. Since the object of this portion of the experiment was to use as many polyps as possible to make measurable amounts of hormone, the failure of some of the animals to segment (and make hormone) indicated that 350 polyps per chamber was too many. We decided, therefore, to use 300 polyps per SCA because our tests showed that they segmented and had undoubtedly made hormone.

Current studies @ 28°C of polyps treated with KI plus radioactive KI indicate that the amount of hormone produced may be greater at a pre-segmentation time period and therefore, the time of induction with KI in flight would need to be later than the formerly proposed MET Day 4.

If-T4 Hormone Isolation, Assay and Identification: After testing numerous extraction methods, we have chosen 80% ethanol, 20 mM NaOH to extract the hormone from *Aurelia*. This solvent mixture has proved to extract and preserve more hormone than acidic ethanol, pronase treatment or a number of other treatments. The alkaline ethanol mixture is homogenized in a glass vessel held at 4°C and the solution centrifuged at 10,000g for 10 minutes. The supernatant is removed and the pellet is re-extracted two more times. The pooled supernatant is then diluted with aqueous HCl to a final concentration of 20% ethanol, 0.3-0.4 M HCl. To extract hormone from ASW, the ASW is adjusted to an identical ethanol, HCl concentration.

The ethanol-HCl mixture is run over 3 ml beds of LH-20 resin, previously treated with 0.1 M HCl, contained in polyethylene columns with glass wool frits. The columns are washed consecutively with three 3 ml portions of 0.1 M HCl, 3 ml of distilled H₂O, 1.8 ml of ethanol:0.1M NH₄OH, aqueous (1:1) and finally, three 3 ml portions of 0.1 M NH₄OH in ethanol. The first two portions of the 0.1 M NH₄OH in ethanol contain the iodinated compounds of interest. These fractions are combined and dried under nitrogen at 40°C.

The compounds are assayed via an RIA or chemiluminescent thyroxine assay using antibodies specific for thyroxine. For compounds other than thyroxine, we utilize an iodine assay based on cerium-bromide displacement with fractions first separated by HPLC. The HPLC conditions are a 250 mm Beckman Ultrasphere C18 column with 5 mM particles in a mobile phase of 40:30:10 (water:methanol:acetonitrile with 5 mM phosphate, pH 3.5 at a flow rate of 1.1 ml. Timed fractions containing the compounds of interest are collected using iodinated standards as a reference.

Fixation for Ultrastructural Studies: In an effort to simulate flight conditions, ephyrae were fixed with various concentrations of glutaraldehyde (1.1 ml in 30 ml of ASW with KI). They were exposed to concentrations of glutaraldehyde ranging from 14% to 70% for either 24h or 48h which would be the time between the injection of the fixative and landing of the shuttle. Glutaraldehyde diluted with sodium cacodylate buffer and without buffer were used for fixation. All of these fixations were followed by post-fixation with 6% glutaraldehyde (simulated post-flight), buffer rinse, osmium post-fix, and tannic acid treatment prior to embedding the samples in Epon. The method of choice was determined to be injection of 1.1 ml of 50% glutaraldehyde (diluted from 70%

glutaraldehyde with sodium cacodylate buffer) followed by the post-fixation treatment described above. This method provided the best fixation of both jellyfish rhopalia (especially hair cells) and muscle.

A simulation was also done in which fixative was injected into an SCA with strobilating jellyfish and the turbulence of the introduced fluid was videotaped. The jellyfish were fixed instantly and were not damaged by the streaming fixative. Likewise a simulation of KI induction which would have occurred in-flight was done in which the KI was introduced into the fixative block and then injected into the SCA containing the jellyfish. The jellyfish were not damaged by the injection.

In another test, polyps scheduled to return alive were placed in an SCA next to an SCA in which glutaraldehyde fixative was introduced. The fixative killed those jellyfish which were to be fixed but when the animals from the adjacent SCA were removed 48h later, they were still alive.

Jellyfish Cultures: This year, we continued to grow large numbers of jellyfish cultures for use for these tests and for the anticipated flight experiment. We have sub-cloned some of the best cultures after testing them for a high level of normality. In addition, we have developed an improved method for growing more jellyfish in aquaria by adding bio-balls to the aquarium providing more surface area for the polyps to attach. We provided hand-care to approximately 200 cultures and we collected new stocks from nature which were to be tested in preparation for the (now-defunct) flight experiment.

This experiment is designed to provide a new understanding of the basic biological processes involved in the utilization of thyroid hormones(s) for development of biological organisms on Earth as well as in space. Thus far, little is known about the effects of thyroid hormone(s) on developing higher organisms in microgravity partly because the time required for completion of mammalian development far exceeds the current time periods of shuttle flights. Tiny jellyfish polyps, however, synthesize a thyroid-type hormone which induces the development of jellyfish of new form, ephyrae, within a week. These organisms provide a rapidly developing model system for the quantitation of the hormone and its receptors(s) in space (and ground controls) during its synthesis and utilization. Through this flight experiment, we will be able to investigate the role that this hormone plays in: (a) the differentiation of new structures such as graviceptors with statoliths and hair cells; (b) the differentiation of a new neuromuscular (motor) system; and (c) demineralization of statoliths. The information gained from these studies will help us to understand the role that the hormone and its receptor(s) play on Earth in the differentiation of similar structures in mammals, including humans. Such an understanding could lead to prevention of hypothyroid or hyperthyroid-related birth defects and to the prevention and/or cure of receptor-based thyroid diseases.

Earlier microgravity research using the jellyfish developmental model indicated that the jellyfish thyroid-type hormone, Jf-thyroxine, may be synthesized in greater amounts in space. If so, then thyroid hormones of mammals may be synthesized in higher amounts, possibly giving rise to a hyperthyroid condition in mammals, particularly those maintained in space for long time periods. If such a hyperthyroid condition were to occur in pregnant mammals in space for long periods, the increased hormone production could impact fetal development. Further, a knowledge of specific detrimental microgravity effects on hormone production and function could lead to the development of countermeasures to prevent such effects in animals (including humans) in space. The jellyfish research, therefore, could ultimately contribute information needed to achieve human long-term occupancy in microgravity on the space station or during long-term space travel.

In addition to the increased understanding of the effects of the thyroid-type hormone in space, a comparison of microgravity effects with ground controls could lead to a better understanding of the role that gravity plays in developing animals on Earth, especially regarding their thyroid hormone and receptor synthesis, distribution, and/or utilization.

FY97 Publications, Presentations, and Other Accomplishments:

Spangenberg, D.B., Coccaro, E., Schwarte, R., and Lattanzio, F "Hair cell and statocyst differentiation in *Aurelia*." (Abstract) ASGSB Bull. 10(1), 34.

Spangenberg, D.B., Coccaro, E., Schwarte, R., and Lattanzio, F. "Hair cell and statocyst differentiation in *Aurelia*." ASGSB annual meeting, Charlotte, NC (October, 1996).

Spangenberg, D.B., Coccaro, E., Schwarte, R., and Lowe, B. "Touch-plate and statolith formation in graviceptors of ephyrae which developed while weightless in space." Scanning Microscopy (In Press).

Photosynthesis and Metabolism of Super Dwarf Wheat in Microgravity

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$90,000

Flight Information:

Experiment ID: 9601269

Flight Assignment: BPS-1 (STS-96 [Target])

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BPS

Task Description:

The photosynthetic rate of higher plants is a critical component of plant-based atmospheric regeneration systems being proposed for long-duration space missions. It is only through direct measurement of photosynthesis in microgravity that an informed decision on the feasibility and design of these systems can be made. The overall objective of this research is to determine the effect of microgravity on photosynthetic response of plant tissues developed in either gravity or microgravity.

The specific objectives of this research are:

- 1) Determine the effect of microgravity on CO₂ and light response curves of Super Dwarf wheat.
- 2) Determine the effect of microgravity on metabolism and electron transport processes associated with photosynthetic and respiratory gas exchange.
- 3) Determine the effect of microgravity on carbohydrate partitioning in Super Dwarf wheat.
- 4) Determine the effect of microgravity on gas exchange, including H₂O, over range of atmospheric vapor pressure deficits.
- 5) Utilize the knowledge gained to understand the response of plants grown under elevated CO₂ conditions of commercial controlled environment crop production systems.

To test the hypothesis that net carbon exchange rates are sustained in microgravity, we propose to measure and characterize the CO₂ and light response curves for canopy photosynthesis of wheat cv. Super Dwarf canopy grown in microgravity. Net carbon exchange and water movement through the canopy will also be measured over defined range of vapor pressure deficits. Post-flight analysis of the tissue for primary photosynthesis parameters, including PSI, PSII, electron transport, and carbohydrate partitioning, will be made and correlated to in-flight data. These measurements of whole canopy gas exchange in microgravity will be used to understand

the effects of microgravity on photosynthesis, to quantify the effects on metabolism, and to model the impact of microgravity on biological approaches to atmospheric regeneration for long-duration space missions.

The Photosynthesis and Assimilatory System Testing and Analysis (PASTA) experiment will obtain direct measurements of photosynthesis and transpiration during the initial hardware verification flight of the Biomass Production System (BPS). Four high fidelity ground support units (GSUs) of the Biomass Production System (BPS) plant growth chambers were obtained in July 1997 and used to evaluate suitability of media for growing wheat in the BPS nutrient delivery system. Super Dwarf wheat was grown on the following four substrates: 1) Arcillite with slow-release fertilizer; 2) Zeolite developed at Johnson Space Center; 3) Commercial zeolite mixture developed through Small Business Innovative Research (SBIR) program by Boulder Innovate Technologies (BIT); and 4) Peat vermiculite mixture. These studies were replicated under a range of controlled environment conditions. Total biomass accumulation and water usage was used to estimate net daily photosynthesis and transpiration rates of plants grown on the various media. Measurements of single leaf photosynthesis and stem water potential were used as physiological indicators of plant response under the conditions being evaluated. Based these measurements, two media were selected for further evaluation: Arcillite with slow-release fertilizer and zeolite mixture developed by BIT.

Mathematical models were developed to predict net gas exchange of Super Dwarf Wheat under environmental conditions and hardware limitations anticipated during space flight conditions. These models are being used to establish test procedures for CO₂ and light response tests under a range of photosynthetic rates, external CO₂ concentrations, and BPS chamber leak rates.

This research will increase our understanding of plant growth and development as affected by space flight conditions. The measurements of gas exchange will provide direct measurements of the fundamental processes of photosynthesis and transpiration critical to life support on long-duration space missions. Taken together, the three dimensions of the experiment (gas exchange, partitioning, and metabolism) will result in a more complete picture of microgravity influences on photosynthesis. The CO₂ and light response curves will allow researchers to establish whether canopy photosynthetic responses are affected by space conditions. This is significant since plants can be used to regenerate the atmosphere in space conditions through removal of potentially toxic CO₂ and production of O₂. In addition, the tests to evaluate movement of H₂O via transpiration are important since they are indicative of stomatal responses which regulate photosynthesis. Further, the impact of microgravity on transpiration is significant since plants can be used to purify water under space flight conditions. These studies will demonstrate the suitability of various solid substrates for supporting plant growth which can be transferred to commercial activities. These studies involving gas exchange at elevated CO₂ concentrations increase our understanding of biological impacts of increasing levels of atmospheric CO₂ on Earth-based ecosystems. Further, our levels of understanding of plant responses under range of CO₂ and light conditions will have potential benefits to commercial controlled environment agriculture industries.

FY97 Publications, Presentations, and Other Accomplishments:

Stutte, G.W. "PASTA: An experimental overview." Kennedy Space Center, FL (September, 1997).

Stutte, G.W. "Photosynthesis and metabolism of super dwarf wheat in microgravity." Ames Research Center, Moffet Field, CA (April, 1997).

Space Experiment on Tuber Development & Starch Accumulation for CELSS

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Funding:

UPN/Project Identification: 199-61-17-24

Solicitation: not available

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 5

Post-Doctoral Associates: 2

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Flight Assignment: USML-2 (STS-73)

Task Description:

Tuber production of white potatoes is of major importance for providing energy-rich carbohydrates in controlled ecological life support systems (CELSS). Starch represents the major source of energy in the edible part of potato tubers, yet existing information implies that accumulation of starch in plant tissues is reduced under microgravity. Thus use of potatoes, and other crops storing large amounts of starch for life support in space, has been questioned, particularly in microgravity of Earth-orbiting stations, but also under reduced gravity on Moon and Mars. This experiment was proposed to study the effect of weightlessness on accumulation of energy-rich starch in potato tubers using excised leaves (explants) on which the axillary buds can be induced to develop small tubers in 10-14 days. This provided an easily controlled system for study of tuber growth and starch production in microgravity on the space shuttle. The experiment was flown in conjunction with the hardware evaluation of the ASTROCULTURE™ plant growing unit developed by the Wisconsin Center for Space Automation and Robotics and included as a middeck locker experiment on the 16 day flight of USML-2 mission in October 1995. The study determined that the tubers formed in the explant system under microgravity had the same gross morphology, the same anatomical configuration of cells and tissues, and the same sizes, shapes, and surface character of starch granules as tubers formed in a 1-G environment. The total accumulation of starch and other energy containing compounds was similar in space flight and ground control tubers. Enzyme activity of starch synthase, starch phosphorylase, and total hydrolase was similar in space flight and ground controls but activity of ADP-glucose pyrophosphorylase was reduced in the space flight tuber tissue. This experiment documented that potatoes will metabolize and accumulate starch as effectively in space flight as on the ground and thus, this data provides the potential for effective utilization of potatoes in life support systems of space bases.

During FY 97, effort was concentrated on completing the manuscripts that detailed the results of the flight experiment and of the ground-based research. This work has been completed except for the final reviews and

submission of a paper on the biochemical analysis of the plant tissue developed in space. A manuscript detailing the ground based studies undertaken to ensure the successful flight experiment was submitted to *Life Support & Biosphere Science Journal* but no decision has been returned concerning its acceptance.

Several presentations were made to local professional groups and to science classes at local schools by all three of the principal investigators. Tibbitts made an invited presentation on this research at the University of New Hampshire in March, 1997. Croxdale presented results of this research at the Midwestern section meeting of the American Society of Plant Physiologists in April, 1997.

Funding for this project ended on September 30, 1997.

The development of functioning CELSS systems, which will involve food production, food processing, and total waste recycling, will provide some exciting technological spinoffs on Earth. Of particular significance should be waste recycling, which needs to be a near-perfect system with no waste accumulation. Transfer of this technology to Earth systems will have some tremendous paybacks and these are already being investigated for Antarctica and remote Alaskan sites.

FY97 Publications, Presentations, and Other Accomplishments:

Brown, C.S., Tibbitts, T.W., Croxdale, J.L., and Wheeler, R.M. "Potato tuber formation in the spaceflight environment." *Life Supp. Biosphere Sci.* (In Press).

Cook, M.E., Croxdale, J.L., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. "Development and growth of potato tubers in microgravity." *Adv. Space Res.* (In Press).

Croxdale, J., Cook, M., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. "Structure of potato tubers formed during space flight." *J. Exp. Bot.* (In Press).

Croxdale, J.L., Cook, M.E., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. "Structural aspects of potato tubers formed in space." (Abstract) *ASGSB Bull.*, 10(1): 13 (1996).

Tibbitts, T.W., Croxdale, J.L., Brown, C.S., and Wheeler, R.M. "Growth and starch accumulation of potato tuber." *USML-2/USMP-3 L+1 Conference Report.* Marshall Space Flight Center, AL (In Press).

Effects of Microgravity on Tobacco Hornworm (Manduca Sexta) During Metamorphosis

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10

Solicitation: 89-13-OSSA IML-2

Initial Funding Date: not available

Expiration: not available

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$40,000

Flight Information:

Experiment ID: 8913078

Flight Assignment: BRIC-04 (STS-70, June 1995); BRIC-07 (STS-77, May 1996)

Responsible NASA Center: Kennedy Space Center

Flight Hardware Required: BRIC

Task Description:

Studies on altered orientation of tobacco hornworms (*Manduca sexta*) pupa relative to gravitational field have shown changes of some amino acids, rate of adult development, and flight muscles. All of these parameters are dependent on ecdysone levels which are elevated by reorienting the insect into a head-up vertical position. The following studies were undertaken to examine the effects of microgravity on tobacco hornworm ecdysone release and subsequent development. Pupae were loaded into passively controlled biological research canisters (BRICs), placed in a shuttle mid-deck locker, and recovered 10 days after launch. Examinations revealed significant development in both flight and ground-control organisms. Though space flight caused development to be slowed by about 15%. Flight pupae lost less weight than controls consistent with slower development. Flight insects required 6% longer time to become moths even though only about half of adult development occurred during space flight. Postflight, the rate of development was normal. Space flight caused a decrease in total amino acids due to a decrease in the concentrations of ile, urea, cys, met, asp, thr and glu+gln. Insects subjected to weightlessness and allowed to complete development showed an 11% lower protein content and a 10% smaller protein concentration of their flight muscle showing that muscle growth was diminished. Hemolymph concentrations of trehalose were not affected by space flight. The timing of this flight study put us past the peak period of ecdysone release. Despite the very low levels of ecdysone in these insects, we detected a trend towards less ecdysone in flight insects. We speculate that without gravity, flow of the hemolymph away from the head region was slower therefore reducing ecdysone availability and slowing development.

During FY97, analysis of the flight samples was completed. Analyses were finalized for hemolymph amino acids, ecdysone, and trehalose. The insects flight muscles were evaluated for mass, protein content and protein concentration. A final report on the flight results was submitted to KSC. We have been able to mostly answer the question of what effect space flight has on adult development of the tobacco hornworm. The need to refly the experiment for a longer duration compromised our ability to obtain meaningful ecdysone data. If we had

been able to demonstrate a significant drop in ecdysone concentration, it would have strongly supported our hypothesis. A question that arises is how space flight leads to effects on development. One possibility is a change in hemolymph flow dynamics. This year's progress will allow us to prepare a manuscript for publication during FY98.

The presence and influence of gravity is taken for granted, yet there are still many basic biology questions which must be addressed concerning the role gravity has played in evolution and the consequences of its constant effects on the development of various living organisms. Metamorphosis provides a biological process which is clearly defined and which can be further examined for its responsiveness to gravity. Laboratory studies have shown that just altering the insect's orientation relative to the gravity vector produces marked metabolic changes. Mammalian studies have already shown marked physiological changes when the influence of gravity is removed. Because mammalian systems are far more complex, a simple model, such as the closed system of the metamorphosing insect, may aid in gaining a better understanding of how subcellular processes respond to and are affected by gravity. Flight experiments are essential in this regard for permitting comparisons between development under normal gravity conditions and the absence of gravity.

FY97 Publications, Presentations, and Other Accomplishments:

Webster-McElvogue, K., Bayomi, S., Ochoa, M., O'Connor, D., Peterson, E., Polanco, R., and Tischler, M.E. "Use of BRIC hardware to study adult development of the tobacco hornworm (*Manduca sexta*)." (Abstract) ASGSB Bull., 10, 48 (1996).

Effect of Spaceflight on TGF- β Expression by hFOB Cells

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Funding:

UPN/Project Identification: not available
Initial Funding Date: 1994
Students Funded Under Research: 0
FY 1997 Funding: \$40,000

Solicitation: 93-OLMSA-04
Expiration: 1997
Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9304007
Flight Assignment: NIH-C4 (STS-69, July 1995); NIH-C6 (STS-80, 1996)
Responsible NASA Center: Ames Research Center

Task Description:

Weightlessness results in skeletal wasting in astronauts. The bone loss is similar to that which occurs in people who undergo prolonged bedrest or, in some cases, lose the use of one of their limbs due to injury or disease. The exact cause of the bone loss is not yet clear but is at least partially due to decreased activity of osteoblasts, the cells which produce the matrix which mineralizes to become bone. Weightlessness results in decreased bone formation in rodents as well as humans. Studies performed on rats implicate a protein which is produced by bone cells and is important in the communication between cells. The gene for that protein was found to be expressed in bone at reduced levels following space flight, but that level was dramatically increased (within 24 hours) when normal activity was reestablished following space flight.

This experiment, flown on STS-69 and STS-80, was designed to determine whether gene expression is altered in cultured bone cells following space flight and if so, how quickly the levels return to normal after flight. Results from this experiment will help us determine the usefulness of cultured bone cells in understanding how the acceleration due to gravity functions to maintain bone cell activity.

The cells to be used in this study are unique. They have been altered to allow them to grow nearly indefinitely at a low temperature (35°C), but when cultured at a higher temperature (39°C), they stop growing and become mature osteoblasts which synthesize bone matrix. This experiment will study the effects of weightlessness and recovery on the mature form of the osteoblast-like cells.

hFOB cells were cultured on cytodex, loaded on a cell culture model system (CCM), and flown on STS-80, a 17-day flight. Flight and ground control cells demonstrated no difference in glucose utilization or in procollagen (carboxyterminal propeptide) and prostaglandin E2 accumulation into the media. These results indicate that space flight had no effect on cell growth and suggest that there was no effect on cell differentiation. The lack of an effect of orbital space flight on steady-state mRNA levels for osteonectin, alkaline phosphatase, IL-12 and

TGF- β 1 supports the conclusion that weightlessness has no effect on hFOB cell differentiation. On the other hand, the mRNA levels for TGF- β 2, IL-1 α , IL-1 β , and IL-6 were transiently diminished following space flight. The altered expression of skeletal signaling peptides suggests that space flight has gene specific effects on bone cells which may influence bone remodeling. The remaining analyses of the hFOB cells flown on STS-80 will focus on cell histology and ultrastructure.

The long-term objectives of this research are to understand the cellular and molecular mechanisms which mediate skeletal adaptation to mechanical usage. Weight bearing is essential to establish and maintain the normal balance between bone formation and bone resorption that functions to achieve and preserve bone volume. Skeletal unweighting, whether due to space flight, prolonged bedrest, paralysis, localized stress shielding following arthroplasty, or cast immobilization, leads to bone loss and an increased risk for fractures. We hypothesize that cyclical mechanical stimulation has direct effects on osteoblasts to modulate expression of one or more signaling peptides (growth factors). In turn, these osteoblast-derived regulatory peptides may act on osteoblasts to regulate bone matrix synthesis, osteoclasts to regulate bone resorption, and on osteoblast and osteoclast progenitors to regulate the proliferation and subsequent differentiation of these cells to osteoblasts and osteoclasts. An exciting aspect of this model is that it identifies a rational means of intervention to prevent disuse osteopenia; it should be possible to mimic the protective effects of weight bearing in the unloaded skeleton by regulating the local levels of the appropriate bone cell derived signaling peptides. The focus of these studies is the TGF- β , an important osteoblast-derived skeletal growth factor whose expression is regulated by weight bearing. We have shown that mRNA levels for TGF- β are reduced in limbs of rats flown in space and quickly revert to normal values following restoration of normal weight bearing. This study seeks to determine whether isolated bone cells in culture respond to the near weightlessness of space flight and return to a 1-G environment in a manner analogous to bone cells in the intact animal. If the manner is affirmative, then cultured bone cells could be used to elucidate the molecular mechanisms mediating regulation of TGF- β expression as well as provide a simple model system for testing the activities of potential pharmacological agents. This line of research may benefit many individuals, because disturbed bone cell signaling plays a role in many osteopenias, including postmenopausal osteoporosis.

Effect of Space Travel on Skeletal Myofibers

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10

Solicitation: 93-OLMSA-04

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 5

Post-Doctoral Associates: 2

FY 1997 Funding: \$0

Joint Agency Participation: NIH/WRAIR

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Flight Information:

Experiment ID: 9304010

Flight Assignment: NIH-C2 (STS-66, 11/94); NIH-C5 (STS-72, 1995); NIH-C7 (STS-77, 5/96)

Responsible NASA Center: Ames Research Center

Task Description:

This experiment will use tissue-cultured muscle cells to study the effects of space flight on muscle atrophy, protein turnover rates, and growth factor secretion to determine whether tissue-cultured skeletal muscle fibers exposed to microgravity will atrophy in the same way as fibers in humans and other animals. The lack of tension on muscles in space due to the lack of gravitational force offers the opportunity to study the cellular mechanisms that cause microgravity-induced atrophy.

This type of research may help identify and develop countermeasures required if people are to sustain muscle strength on long-duration space voyages. The experiment will also provide a rapid screening system for testing drugs to prevent muscle atrophy.

The Middeck Space Tissue Loss Module developed by the Walter Reed Army Institute of Research was utilized to determine whether microgravity directly affected tissue cultured skeletal muscle cells. Avian bioartificial skeletal muscles containing differentiated skeletal myofibers and connective tissue fibroblasts were flown aboard the Space Shuttle (Space Transportation System, STS) on Flights STS-66 and STS-77. Assays for cellular metabolism, total protein synthesis, and protein degradation rates were performed. The effects of reloading the muscle cells post-flight were also determined by measuring total protein synthesis rates and myosin heavy chain, collagen, and fibronectin accumulation and synthesis following return to Earth. Overall STS-77 results compared well with those obtained in the STS-66 experiment and to changes seen by others in skeletal muscle of rodents flown aboard the Space Shuttle. In both tissue culture and animal systems, neither general muscle metabolism rates nor total protein degradation rates are significantly altered by space flight, while muscle fiber size and total protein synthesis rates are reduced in flight relative to ground controls. A preferential decrease in myofibrillar content occurs in animals and tissue cultured muscle. Finally, reloading of the muscle cells after

flight activates the protein synthetic process both *in vitro* and *in vivo*. The results from these two flight experiments show for the first time that space travel has a direct effect on skeletal muscle cells separate from any systemic effects resulting from altered circulating growth factors.

While the primary goal of this project is to understand and treat space travel-induced skeletal muscle atrophy, the results from these studies may have applications for several skeletal muscle wasting disorders on Earth. These include the severe muscle wasting observed in paralyzed patients and in the frail elderly, both of which partially respond to the increased tension associated with exercise and physical therapy. By better understanding the interactions of microgravity and muscle atrophy, optimization of physical therapy could be optimized for increased patient mobility and independence.

FY97 Publications, Presentations, and Other Accomplishments:

Payumo, F.C., Kim H., Sherling, M., Smith L., Keeping, H.S., Valentini, R., Drozdoff V., and Vandenburg, H.H. "Expression of recombinant human bone morphogenetic protein-6 in C2C12 myoblasts." (Abstract) *Artif. Organs*, 21, 496 (1997).

Powell, C., Shansky, J., and Vandenburg, H.H. "Engineering of human bioartificial skeletal muscle for delivery of therapeutic molecules." (Abstract) *Artif. Organs*, 21, 496 (1997).

Shansky, J., Del Tatto, M., Chromiak, J., and Vandenburg, H. "A simplified method for the *in vitro* tissue engineering of skeletal muscle organoids." *In Vitro Cell. Dev. Biol.* (In Press).

Vandenburg, H.H., Chromiak, J., Yamada, S., Del Tatto, M., Shansky, J., and Goldstein, L. "Tissue engineering skeletal muscle organoids secreting rhGH attenuate skeletal muscle disuse atrophy." *Keystone Symposia on Molecular and Cellular Biology of Gene Therapy*, Snowbird, UT (April 13 - 19, 1997).

Vandenburg, H.H. "Tissue engineering striated muscle organs by mechanical forces." (Abstract) *ASME Transactions*, 1 (1996).

Vandenburg, H.H. "Direct effect of space travel on skeletal myofibers." *Fifth World Congress International Society for Adaptive Medicine*, Framingham, MA, 59 (September 7 - 10, 1997).

Vandenburg, H.H., Chromiak, J.A., Shansky, J., and Del Tatto, M. "Effects of space travel on cell metabolism and protein turnover of skeletal muscle cells." *Am. Soc. Cell. Biol. Mtg. - Special Session*, H59 (1996).

Vandenburg, H.H., Chromiak, J., Shansky, J., and Del Tatto, M. "Initial International Space Station (ISS) definition studies for examining the effects of long-term space travel on tissue cultured mammalian skeletal myofibers." (Abstract) *ASGSB Bull.*, 10, 52A (1996).

Vandenburg, H.H., Del Tatto, M., and Shansky, J. "Bioartificial skeletal muscle as a platform for long term delivery of growth hormone." (Abstract) *Artif. Organs*, 21, 534, (1997).

Vandenburg, H.H., Del Tatto, M., Shansky, J., LeMaire, J., Chang, A., Payumo, F., Lee, P., Goodyear, A., and Raven, L. "Tissue engineered skeletal muscle organoids for reversible gene therapy." *Human Gene Therapy*, 7, 2195-2200 (1996).

Vandenburg, H.H., Del Tatto, M., Shansky, J., LeMaire, J., Chang, A., Payumo, F., Lee, P., Goodyear, A., and Raven, L. "Tissue engineered skeletal muscle organoids for reversible gene therapy." (Abstract) *In Vitro* 32, 53A, (1996).

Vandenburg, H.H., Shansky, J., Del Tatto, M., and Chromiak, J. "Methods for the organogenesis of skeletal muscle in tissue culture" in *Methods in Tissue Engineering, Methods in Molecular Medicine Series.* Edited by: Morgan, J. and Yarmush, M. Humana Press (In Press).

Functional Development in a Model Vestibular System

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 106-50-10
Initial Funding Date: not available
Students Funded Under Research: 3
FY 1997 Funding: \$ 190,000

Solicitation: 93-OLMSA-07
Expiration: not available
Post-Doctoral Associates: 2

Flight Information:

Experiment ID: 9307510
Flight Assignment: ARF-3 (TBD)
Responsible NASA Center: Kennedy Space Center
Flight Hardware Required: ARF

Task Description:

Development of the gravity-sensing organs in altered gravity conditions has been studied in several series of experiments, with variable and often conflicting results reported. On IML-2 (STS-65) we flew pre-fertilized eggs of the Japanese red-bellied newt, *Cynops pyrrhogaster*, staged such that they would not produce any otoconia (the dense "stones" in the inner ear, upon which gravity exerts its force) before they reached μ -G. Newt eggs offer a favorable preparation in which to study vestibular-system development since their inner ear progresses from very primitive stages to a nearly adult form in the 16-day time available for a space shuttle flight. Our original hypothesis was that growth of the otoliths would be affected by the G field, with larger otoliths being formed in μ -G partially compensating for the decreased weight of a given mass in μ -G. In the week after shuttle landing, the utricular and saccular otoliths were of nearly identical volume in the newt larvae reared in μ -G and in the ground-control larvae. However, amphibians possess a second system of otoconia, produced in the endolymphatic sac (ES). The ES communicates with the saccule via the endolymphatic duct (ED). In the flight-reared newts, the volume of otoconia in the ES was 3 to 4 times larger than in ground-control animals at the same developmental stage. At later stages, when the otoconia from the ES move through the ED to the saccule, these additional stones cause the saccular otolith to be larger in the flight-reared larvae. We used a Japanese X-ray micro-focus system to follow growth of the otoliths in one flight-reared larva for 9 months after return. The saccular otolith volume was significantly larger in this animal, compared to several ground-control animals from the same batch of eggs, for 5 months post-flight, with the largest difference seen at 2 months post-flight. This had behavioral consequences in that the flight-reared larva exhibited abnormal head position, with the head raised 30° from the horizontal for its entire terrestrial life (normal newts keep their head horizontal).

In experiments performed in Japan using parabolic flight, we identified stereotypic behavioral responses to transient exposure to μ -G at different developmental stages which correlated with the development of different portions of the vestibular system. Using the Canadian Aquatic Research Facility (ARF) we will rear additional newt larvae in small (35ml capacity) aquaria. One set will be reared at μ -G by having the centrifuge rotate at only one revolution per ten seconds (to equalize environmental conditions among the six arms of the centrifuge), and the other set will be reared at 1-G by having the centrifuge run continuously at 80 RPM. Video facilities are available to observe behavioral responses to the first exposure to μ -G, produced by briefly stopping the 1-G centrifuge and observing the response to the first exposure to 1-G, produced by rotating the quasi-static centrifuge at 80 RPM for several minutes late in the flight.

Measurements of the otolith-ocular reflex were made in flight- and ground-reared larvae from IML-2. Although the techniques used at that time were not fully adequate, the results indicate that the gain of the reflex increases with developmental stage in ground-reared larvae, as reported by others. In the flight-reared larvae, there was considerable variability in the measured gain, but there was no indication of a systematic change in gain with development. This indicates that the reflex by which the animal uses gravitational stimulation of the otoliths to stabilize an image on the retina does not develop normally in the absence of gravity. Improved measurement techniques are being developed for use on ARF-3.

In summary, the main aspects of the IML-2 experiment will be replicated using a facility that allows in-flight 1-G controls. Approximately half of the larvae will be maintained for several months after flight in an attempt to verify the indication that the greatest changes in both the endolymphatic and saccular otoconia occurred after the larvae were introduced to 1-G conditions.

Experiments have continued to determine the maximum number of fertilized newt eggs that can be maintained in the ARF Specimen Container Units (SCUs). With 10 larvae in each 35 cc aquarium (SCA), all larvae survived for 30 days in two runs and for 10 days in another two runs. With 15 larvae in a SCA, the survival is reduced to approximately half for runs of 13 to 30 days. If one larva in an SCA dies, the others in the same SCA are likely to succumb within approximately 5 days. Thus, for a ten to twelve day shuttle mission, we can accommodate 10 (20 per SCU), but not 15 larvae per SCA. We expect to fly 3 SCUs in both the 0-G and 1-G portions of the ARF. Further tests will be run with 12 larvae per SCA.

Additional studies of the ultrastructure and development of the newt endolymphatic sac (ES) have been completed, in collaboration with Dr. Gao. One of these is currently in press (Gao, Wiederhold and Hejl, cited below) and two more papers are in revision to accommodate reviewers' suggestions. The ES is of particular interest since the greatest differences between flight- and ground-reared newt larvae were seen on IML-2 in the production of the aragonitic otoconia in the ES. Distinct changes in the nature of the junctions between cells comprising the wall of the ES and duct have been identified, which appear at the time that production of the ES otoconia begins. With specimens developed in micro-gravity on the ARF-3 flight, we will look for earlier appearance of these specializations to correlate with the expected increased production of endolymphatic otoconia in space-reared larvae.

Dr. Glenn Harper, a Neurosurgery resident, has begun his year-long research rotation in our laboratory. He has designed a new system to measure eye movements in newt larvae during rotation about the body axis, as a measure of the otolith-ocular reflex (OOR). A special optical-glass chamber to hold the larvae on a rotating table, with a CCD camera focused on the eye, is currently being constructed. This system will be used to measure the OOR in both flight- and ground-reared larvae. This will allow us to confirm and better document the preliminary results from IML-2, indicating that the reflex does not develop normally in animals reared in the absence of gravity. The gain of the OOR increases with developmental stage in newt larvae reared on Earth, as has been reported in *Xenopus* larvae. However, the gain of the reflex fails to change with stage in the newt larvae reared in micro-g. This indicates that if there is not gravity to activate the otolith system when the synaptic connections mediating this reflex are established, the reflex fails to develop normally.

It is well known that animals and man lose calcium from their bones during extended periods in space. Our studies are designed to help understand what processes control biomineralization. There is growing evidence that the lack of gravity can adversely affect bone mineralization even in isolated embryonic bones. Thus, there appears to be a fundamental interaction between mineralization and gravitational forces. Such an interaction could have major consequences in a developing gravity-sensing organ which depends on the gravitational force on a dense calcified mass to activate sensory receptor cells. Our studies will address both the formation of the "test mass" in microgravity and the ability to develop gravity-related reflexes in the absence of gravity. Since normal development on Earth always occurs in a 1-G field, the effect of that gravity has largely been neglected. If the otolith-ocular reflex were to develop abnormally in μ -G, this could imply abnormal development if animals were maintained in an altered position during critical developmental stages on Earth.

There is evidence that the otoconia in elderly humans can become decalcified, which would cause a decreased mass with which to sense gravitational and linear-acceleration forces. This has been suggested to contribute to unsteadiness in elderly humans. Our studies of the mechanisms by which otoconia are mineralized will help to clarify the processes of demineralization as well.

Another pathological condition in humans, Benign Paroxysmal Positional Vertigo (BPPV) occurs when otoconia, usually restricted to the utricle and saccule, become attached to the cupula overlying the sensory hair cells in the semicircular canals which sense angular acceleration. The cupula is normally neutrally buoyant in the endolymph, but when it becomes loaded with dense otoconia, it will "sink," giving the patient the sensation that he is spinning, as would be the case during normal stimulation of the semicircular canal. The prevailing hypothesis is that the "extra" otoconia on the cupula have become dislodged from the saccular or utricular otoliths. However, if those otoconia were to be formed by some abnormal chemical condition in the endolymph, similar to the formation of otoconia in the ES crypts in the newt, this would suggest a more specific etiology for BPPV which might be treatable medicinally. We have been in contact with several neuro-otological surgeons who perform surgery to block the semicircular canals in BPPV subjects. If we could obtain some otoconia from such subjects, we can test their crystallography, using Fourier Transform InfraRed spectroscopy. The otoconia in the utricle and saccule are made of calcium carbonate in the calcite crystal form, whereas those formed in the ES in the newt are CaCO_3 in the aragonite crystal form. If otoconia in the semicircular canals of BPPV patients were to be composed of aragonite, this would strongly suggest that they are not displaced otolith otoconia and could be formed by a mechanism similar to that found in the newt.

FY97 Publications, Presentations, and Other Accomplishments:

Gao, W., Wiederhold, M.L., and Hejl, R. "Development of the statocyst in the freshwater snail *Biomphalaria glabrata* (*Pulmonata*, *Basommatophora*)." *Hear. Res.*, 109, 109-124 (1997).

Gao, W., Wiederhold, M.L., and Hejl, R. "The structure of the statocyst of the freshwater snail *Biomphalaria glabrata* (*Pulmonata*, *Basommatophora*)." *Hear. Res.*, 109, 125-134 (1997).

Gao, W., Wiederhold, M.L., and Hejl, R. "Ultrastructure of the endolymphatic sac in the larva of the Japanese red-bellied newt *Cynops pyrrhogaster*." *Cell Tiss. Res.* (In Press).

Gao, W.Y., Wiederhold, M.L., and Hejl, R.H. "The structure and development of the statocyst in the pond snail *Biomphalaria glabrata*." Abstracts of the Twentieth Midwinter Research Meeting of the Association for Research in Otolaryngology 20:38 (1997).

Gao, W.Y., Wiederhold, M.L., and Hejl, R.J. "The structure and development of the statocyst in the pond snail *Biomphalaria glabrata*." (Poster) Twentieth Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, FL (February 2 - 6, 1997).

Gao, W.Y., Wiederhold, M.L., and Hejl, R.J. "The structure and development of the statocyst in the pond snail *Biomphalaria glabrata*." (Poster) Twentieth Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, FL (February 2 - 6, 1997).

Pedrozo, H.A., Schwartz, Z., Dean, D.D., Harrison, J.L., Campbell, J.W., Wiederhold, M.L., and Boyan, B.D. "Evidence for the involvement of carbonic anhydrase and urease in calcium carbonate formation in the gravity-sensing organ of *Aplysia californica*." *Calc. Tiss. Int.*, 61, 247-255 (1997).

Pedrozo, H.A., Schwartz, Z., Dean, D.D., Wiederhold, M.L., and Boyan, B.D. "Regulation of statoconia mineralization in *Aplysia californica* *in vitro*." *Connect. Tiss. Res.*, 35 (1-4), 317-323 (1997).

Wiederhold, M.L. "Development of gravity-sensing organs in microgravity." International Space Station Symposium. Twelfth Annual Meeting for the American Society for Gravitational and Space Biology, Charlotte, NC (October 24, 1996).

Wiederhold, M.L. "Development of gravity-sensing organs in the absence of gravity: Newts in space!" George F. Fried Seminar Series. Department of Biology, Brooklyn College of the City University of New York, NY (October 18, 1996).

Wiederhold, M.L. "Supersonic snails." Department of Otolaryngology-Head & Neck Surgery Alumni Day, The University of Texas Health Science Center, San Antonio, TX (June 14, 1997).

Wiederhold, M.L. "Development of gravity-sensing organs in microgravity." American Society for Gravitational and Space Biology. ASGSB Bull. (In Press).

Wiederhold, M.L., Gao, W.Y., Harrison, J.L., and Hejl, R. "Development of gravity-sensing organs in altered gravity." ASGSB Bull., 10, 91-96 (1997).

Wiederhold, M.L., Gilpatric, K.D., Hejl, R.J., Koike, H., and Nakamura, K. "Development of otoliths and endolymphatic otoconia in newt larvae reared in microgravity." Abstracts of the Nineteenth Midwinter Research Meeting of the Association for Research in Otolaryngology 19:145 (1996).

Wiederhold, M.L., Hejl, R.H., Harrison, J.L., Gao, W., and Hortick, C.H. "Development of a molluscan gravity-sensing organ in microgravity." Fifteenth Annual Houston Conference on Biomedical Engineering Research. The University of Houston, Houston, TX (February 13 - 14, 1997).

Wiederhold, M.L., Hejl, R.H., Harrison, J.L., Gao, W., and Hortick, C.H. "Development of a molluscan gravity-sensing organ in microgravity." Fifteenth Annual Houston Conference on Biomedical Engineering Research. The University of Houston, Houston, TX (February 13 - 14, 1997).

Wiederhold, M.L., Pedrozo, H.A., Harrison, J.L., Hejl, R., and Gao, W. "Development of gravity-sensing organs in altered gravity conditions: Opposite conclusions from an amphibian and a molluscan preparation." *J. Grav. Physiol.* (In Press).

Individual Susceptibility to Post-Spaceflight Orthostatic Intolerance: Contributions of Gender-Related and Microgravity-Related Factors

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Funding:

UPN/Project Identification: 106-50-01
Initial Funding Date: 1997
Students Funded Under Research: 0
FY 1997 Funding: \$165,000

Solicitation: 96-OLMSA-01
Expiration: 2000
Post-Doctoral Associates: 0

Flight Information:

Experiment ID: 9601051
Flight Assignment: TBD
Responsible NASA Center: Johnson Space Center

Task Description:

The long-term objectives of this proposal are unified by the overriding hypothesis that the following factors contribute to the occurrence of postflight orthostatic hypotension in some, but not all, astronauts: 1) gender-related differences in autonomic regulation of arterial pressure; and 2) space flight-induced changes in autonomic regulation which precipitates orthostatic hypotension in predisposed individuals. There are two specific aims. The first is to test the hypothesis that greater orthostatic intolerance in women is caused by lower vasoconstrictive responses that are estrogen-related. The second aim is to test the hypothesis that an abnormality in the central nervous system, caused by space flight, contributes to autonomic dysfunction and orthostatic intolerance. A ground-based study will include premenopausal women at different phases of their menstrual cycle; postmenopausal women before and after estrogen therapy; and men. All groups will undergo an upright tilt test before and after cardiac blockade to test the relative susceptibilities of those who are primarily heart rate responders and those who are primarily vascular responders. Levels of estrogen, catecholamine responses, and alpha-adrenergic receptors will be measured, as well as cardiovascular responses. A flight-related study will measure responses to upright posture, a tyramine pressor test, and a phenylephrine pressor test. Norepinephrine responses to the tyramine test will indicate if there is a central abnormality in signaling. Pressor responses to phenylephrine will indicate any upregulation of alpha1-adrenergic receptors. Levels of estrogen, catecholamine responses, alpha-adrenergic receptors will be measured, as well as cardiovascular responses. As stated in the NRA, "the current goals of the Space Physiology and Countermeasures Program are: to identify and characterize the physiological changes in humans.... and determine mechanisms of these changes.... Cardiovascular responses to microgravity and on return to Earth's gravitational forces must be understood." This project directly addresses those important goals by studying the preflight and postflight interactions of the endocrine and autonomic nervous systems and their relative influences on arterial pressure control on astronauts.

This project was a new start in FY97. Progress during that period was limited to planning and preparation for initiating an experimental study in FY98. The experimental protocol was defined and approved by NASA Johnson Space Center's Institutional Review Board (IRB).

Astronauts experience autonomic dysfunction during and after space flight. However, they recover spontaneously, without intervention. Earthbound patients who suffer from autonomic dysfunction never recover. If we can obtain an understanding of the mechanisms of the onset of and recovery from the space flight-induced dysfunction, it could have important consequences in the diagnosis and treatment of clinically devastating autonomic dysfunction.

Compact, Rapid Response Optical Air Quality Monitor

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Funding:

UPN/Project Identification: 199-04-17-19

Solicitation: 95-OMSA-01

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The proposed program will develop and demonstrate a compact, rapid response air quality monitor for multiple gaseous contaminants. This monitor meets an identified need in advanced technology development for enclosed human environments in space. The sensor technology is based on miniature, room-temperature diode laser absorption in the near-IR between 1.3 and 3.3 μ m. Capability already under development at Physical Sciences Inc. (PSI) for CO, CO₂, NH₃, NO₂, NO, and HF detection will be augmented to include sensitive detection of volatile hydro/halocarbon compounds such as acetone, benzene, cyclohexane, freons, ethanol, propanol, chlorodifluoromethane, dichlorobenzene, and ethyl acetate. The volatile organic compounds (VOCs) will be detected using vibration overtone/combination band absorption near 1.75 μ m. In the first year, a broadly tunable external cavity diode laser will be used to complete spectral surveys of target VOCs to quantify absorption strengths and potential interferences in order to identify optimum spectral regions for selection of monolithic, fiber-coupled diode lasers. In the second and third years, a prototype flight version will be developed and tested. This prototype will use multiple, fiber-coupled lasers to detect multiple species in a compact, light-weight, low-power consumption electro-optic package with no moving parts. AlliedSignal will work with PSI as a subcontractor to assist in the prototype development and the preliminary design of a flight package.

Progress between October 1996 and September 1997 centered on quantitative measurements of the fundamental absorption strengths of selected VOCs in the 1 to 3 micron wavelength region. A near-IR optimized FTIR was used to obtain absorption cross-sections for acetone, benzene, methanol, toluene, 1,2 di-chloroethane, and 1,1,1 tri-chloroethane. Diode laser measurements of methanol were also demonstrated. Initial lasers from JPL operating near 1.7 microns have been evaluated for use in sensors for selected VOCs. An early insertion technology demonstration effort has been identified in support of the NASA Early Human Testing Initiative (EHTI). PSI will bring a prototype sensor for simultaneous measurements of CO₂ and H₂O to the Phase III tests at the NASA JSC LSSIF.

The progress during the past year has included numerous detection "firsts" regarding an important class of compounds in space-borne and terrestrial industrial habitats. Many of the compounds on the NASA Environmental Monitoring Requirements Document list include common industrial solvents whose

concentration are regulated in work environments. PSI envisions commercial markets for the technology developed as a part of this program and has already begun commercialization of several individual gas sensors. The volatile organic compounds are also representative of unburned hydrocarbons from fossil fuel combustion and we expect additional applications in this market as well.

FY97 Publications, Presentations, and Other Accomplishments:

Allen, M.G., Kessler, W.J., and Sonnenfroh, D. "Room temperature diode laser monitors for spacecraft air quality." Paper No. 97-2392, 27th SAE International Conference on Environmental Systems (July, 1997).

Kessler, W.J. and Allen, M.G. "Near-IR diode laser monitoring of volatile organic compounds." Paper CFN2, Optical Society of America Conference on Lasers and Electro-Optics (CLEO) (May, 1997).

Microbial Monitoring Based on Quantitative PCR

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Funding:

UPN/Project Identification: 199-04-17-22

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 189,744

Task Description:

The monitoring of spacecraft life support systems for the presence of health threatening microorganisms is paramount for crew well being and successful completion of missions. Currently most environmental samples are assayed using conventional microbiology techniques that require skilled technicians and elaborate culture media, and sometimes it is days before results are obtained.

The union of the molecular biology techniques of DNA probe hybridization and polymerase chain reaction (PCR) offers a powerful method for the detection, identification, and quantification of microorganisms. This technology is theoretically capable of assaying samples in as little as two hours with specificity and sensitivity unmatched by any other method. This probe-hybridization/PCR has recently come of age in a technology called TaqMan™, invented by Perkin Elmer. Instrumentation using TaqMan concepts is evolving towards devices that can meet NASA's needs of size, low power use, and simplicity of operation. The chemistry and molecular biology needed to utilize these probe-hybridization/PCR instruments must evolve in parallel.

Our project will establish the chemical and molecular biological tools necessary to use the emerging TaqMan technology for monitoring environmental microbes. In a collaboration between the University of Alabama at Birmingham, Perkin Elmer, and NASA's Marshall Space Flight Center (MSFC), we will develop methods using both commercially available as well as Perkin Elmer's next generation Taqman instrumentation, and then test the new methods on water recycled by the MSFC water reclamation system. We will create sets of PCR primers and TaqMan probes that can specifically detect bacteria, fungi, protozoa, and viruses belonging to a list of microbial species and groups we believe could be detrimental to a spacecraft environment. We will establish optimal biochemical methods for sample preparation that would be amenable to the kind of fully automated instrumentation space utilization will require, as well as detailed PCR protocols for quantitative microbial detection.

In addition to space utilization, a microbial monitor will have tremendous terrestrial applications. Analysis of patient samples for microbial pathogens, testing industrial effluent for biofouling bacteria, and detection of biological warfare agents on the battlefield are but a few of the diverse potential uses for this technology.

1. Reagent design: Using computer-based methods we have designed sets of TaqMan PCR primers and probes for the detection of the bacteria, fungi, parasites, and viruses we believe an ISS monitor of water microbiology should detect.
2. Sample preparation: We have developed sample preparation methods that are adequate for isolation of DNA and RNA from bacteria and viruses for subsequent analysis in using TaqMan PCR. To date we have found that no one sample preparation method works for all microbes.
3. Assay specificity, sensitivity, and quantitation: We already have developed and are testing TaqMan PCR assays for these specific groups of bacteria and protozoan parasites: any bacteria, Coliform bacteria, *Staphylococcus sp.*, *Legionella sp.*, *Listeria sp.*, *Giardia sp.*, and *Cryptosporidium sp.* The genus specific assays have a limit of detection of 5-10 organisms. The universal bacteria assay limit of detection is unclear because all commercial preparations of the Taq DNA polymerase used in these PCRs are contaminated with bacterial DNA. Additionally, we have developed an assay for picornaviruses with which we use poliovirus as a model detection organism.
4. PCR Inhibitors: We were concerned that there could be inhibitors of the enzymes used in PCR in the recycled water generated by the ISS water reclamation system being developed at NASA MSFC. We conducted experiments to test this and found no PCR inhibition.
5. New instruments: Assay development in this project has been done using a PE-Applied Biosystems 7700 quantitative PCR instrument. We have begun testing our assays, which were developed on this large laboratory instrument, on a new prototypic micro-PCR system. This micro-PCR system has the small size and low power consumption characteristics necessary for deployment in space, and still has the same capacity to detect microorganisms as the PE Applied Biosystems 7700. Experiments with the prototype micro-PCR instrument using silicon-based microstructures that contained 5 μ l PCR reactions showed it could detect a single copy of bacteriophage lambda DNA in 30 minutes (the lambda phage experiment was not done as part of this NASA project).

The world needs better ways of monitoring clinical, environmental, and industrial samples for microbial contaminants. The aspiration of designers of gene-based microbial diagnostics technology is small, inexpensive, fully automated devices that could rapidly describe the microbial population of any sample of interest. Such an instrument would have applications in every hospital, clinic, water processing plant, and chemical lab in the US. It might even be in every home so that people could test their food for pathogenic bacteria, or know if a child had a strep throat or Lyme disease before you went to see a doctor. Our research would not make that machine; however, it would take a significant step towards that ambition.

FY97 Publications, Presentations, and Other Accomplishments:

Glass, J.I., Wechser, M., Taylor, T.B., Lefkowitz, E.J., Roman, M.C., Albin, M., Paszko-Kolva, C., and Cassell, G.H. "Gene-based detection of microorganisms in environmental samples using PCR." SAE Technical Paper Series (#972424), 27th International Conference on Environmental Systems, Lake Tahoe, NV (July 14 - 17, 1997).

An Advanced Approach to Simultaneous Monitoring of Multiple Bacteria in Space

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Funding:

UPN/Project Identification: 199-04-17-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 1994

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$303,645

Task Description:

The primary objective of the proposed ground-based technology development program is the development of a novel microchip-based microbial analyzer capable of simultaneously detecting, quantitating, and identifying multiple microorganisms found in a space environment. Successful technology development will result in a miniaturized, automated microbial analysis system capable of rapidly monitoring air and water supplies, as well as identifying particular pathogens in the mission environment.

Fast microbial analysis can likely be achieved due to the avoidance of standard cell cultivation procedures which require days to perform. Moreover, the proposed highly sensitive direct CCD detection procedure, combined with the inherent amplification property of rRNA, will likely reduce the combined sample preparation, assay, and detection time from days to hours. Simultaneous microbial monitoring can likely be achieved due to the high density CCD arrays that can support hundreds of immobilized probes per cm² to facilitate multiple microorganism detection and identification in a high throughput manner (1M pixels/sec). Minimal equipment is likely since the probe-based assay is integrated with the miniature CCD detection device, thereby alleviating traditional macro-detection techniques such as epifluorescent and confocal microscopy.

Testing and evaluation of the prototype microbial hybridization detector on RNA extracted from *E. Coli* and *V. proteolyticus* was completed. Specifically, the proximal CCD system detected, imaged, and quantified the RNA mixture within a total of 51 seconds. A detailed paper describing the system performance was published.

The next system evaluation involves the simultaneous detection of numerous microorganisms known to be present in water samples taken from the Lunar-Mars Support Test Project. Here several microorganism probes designed in FY97 will be arrayed in FY98 for testing the processed water obtained from the September 1997 closed environment mission at JSC.

Finally, the capillary DNA array printer was refined to yield higher density arrays by using smaller capillaries and a higher density manifold template. The printer is presently capable of printing 96 different DNA probes in a 1 cm² array area within one second.

The primary objective of the microbial analyzer is to provide a miniaturized, automated microbial analysis system capable of rapidly monitoring air and water supplies, as well as identifying particular pathogens in mission environment. The research would have a far reaching effect on monitoring the environment for manned missions to Mars and other planets in the 21st century from the orbiting space station. The highly sensitive proximal CCD detection procedure would also provide an ideal platform to support automated, low cost DNA sequence analysis for diagnostic applications on Earth. Moreover, the microbial analyzer would be very suitable for routine monitoring for water treatment facilities and hospitals due to its high sensitivity and miniature format.

FY97 Publications, Presentations, and Other Accomplishments:

Eggers, M.D. "Evolution of microarrays: From concept to commercialization." Gordon Research Conference on Bio/analytical Sensors, Henniker, NH (July 27 – August 1, 1997).

Eggers, M.D. "Microarray fabrication and microdetection for diagnostics." IBC 3rd Annual Conference on Biochip Arrays, San Diego, CA (March 5 - 6, 1997).

Eggers, M.D., et al. "Advanced approach to simultaneous monitoring of multiple bacteria in space." 27th International Conference on Environmental Systems, Lake Tahoe, NV (July 14 - 17, 1997).

Advancement in Determining Hazardous Volatile Organic Compounds in Air

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Funding:

UPN/Project Identification: 199-04-17-13

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 151,958

Task Description:

During the last several years, an advanced technology for air monitoring featuring low power, small size, light weight, and high reliability has been transformed through NASA funding from a niche application in military venues to a proven tool for detecting a broad range of hazardous volatile organic compounds in the air of manned spacecraft. Findings on this technology, ion mobility spectrometry, suggest that all the necessary or desired components of a robust and sophisticated chemical analyzer now exist in various configurations. Still missing are a few essential facets needed to move the technology from a potentially useful condition to a completely functional and user-transparent condition. These items center largely on the artificial intelligence of handling analyzer spectra and on the understanding of certain foundation principles including a comprehensive model for the molecular basis of response. The objective of the effort proposed is to advance ion mobility spectrometry to a first generation of fully integrated (i.e., automated) condition involving software for automated identifications of vapors, standardized databases, and predictive capabilities for unknown or unprogrammed vapors through an improved understanding of the foundations of response.

Three major milestones were accomplished in FY97: a) the first successful training of a neural network using whole spectra with an expansive list of chemicals; b) the first comprehensive model to link molecular structure with ion mobility spectrum (i.e., elucidation of the foundation of IMS spectra); and c) the creation of instrumentation fitted with control over all parameters (except ambient pressure) for constructing a data base for over 150 chemicals at multiple temperature and moisture values. Each of these are described below.

Neural Network Training. During FY97, an approach to training a neural network (NN) with whole spectra was successfully demonstrated. In this approach, eight to fifteen spectra for a single chemical spanning a range of concentrations were used to train a NN in contrast with previously unsuccessful NN training exercises where only a single or a few spectra for a chemical were utilized. Since IMS spectra show a concentration dependence, this facet of response was exploited as part of the learning experience for the NN and provided additional detail for training. Spectra in a test group showed better than 93% correct assignment to the proper chemical class (ketone, aldehyde, alcohol, etc.). Throughout this exercise, detailed studies on the influence of spectral preprocessing were made and the findings now provide guidelines for criteria on this essential step for NN training. In summary, the procedures now exist for pre-processing whole IMS spectra and training neural

networks for automated identifications. A second part to a total artificial intelligence for automated operation of IMS is assignment of specific chemical identity. This was attained with 85% correct assignment with a first NN training exercise and is under further development.

Predictive/Interpretative Model for IMS. In FY97, the first comprehensive model to explain the origins of IMS spectra was formulated and experimental results were reviewed and finalized in support of this model. This model now allows the prediction of the qualitative appearance of spectral profiles in IMS (a quantitative model is under development). In this model, gas phase ions are created in the ion source region of the drift tube under steady state conditions so long as ions and sample neutrals co-exist. The ions created are governed by temperature, vapor concentration, and molecular identity and consist of a mixture of cluster ions under common conditions. Once the mixture of ions is extracted by an electric field into a clean gas environment and injected into the drift region, kinetics of ion decomposition become pre-eminent in controlling the ultimate appearance of an IMS spectrum. Ion clusters with three or four molecules attached to the ion rapidly decompose for all chemicals. Proton-bound dimers decompose in the IMS drift time scale and, thus, proton-bound dimers appear for some chemicals (most oxygenated chemicals) and do not appear for others (some amines, alkanes, aromatics, and aliphatics). The simplicity of IMS spectra with protonated monomers and proton-bound dimers are associated with gas phase instability of cluster ions that exist in the source in a mixture that is comprised of several ion clusters as governed by experimental parameters.

New Data Base. At the core of this research program is a study of essential parameters of moisture and temperature on ion mobility spectra for over 150 organic chemicals at various concentrations. A prerequisite of this effort has been the creation of a GC/IMS instrument where the IMS detector is provided with continuously monitored gas with controlled moisture levels. The apparatus for this was completed in FY97 and studies were initiated. The database is expected to be comprised of five to six temperature settings with five to six moisture settings at each temperature for about 160 chemicals. Thus, a 5x5 or 6x6 matrix for 160 chemicals with 6-10 spectra per chemical is being created. This database will be used both for interpretations and for elucidating the influence of moisture and temperature on IMS spectra with relationship to molecular structure.

This research program concerns the detection and identification of toxic or hazardous chemicals in air and consequently has no direct relief of disease or maladies for humans on Earth or in space. However, the discovery of the presence or source of chemical contamination in air often represents the first step in solving a contamination episode or in alerting astronauts in confined quarters of the potential threat to health. As such, a goal of this research program is directed towards eliminating or minimizing the opportunities for inhalation poisoning or for unwelcome inhalation of particular chemicals. This should be regarded as a component for direct relief of diseases on Earth.

One of the most significant trends in chemical instrumentation during the last decade has been the movement toward instruments that can be brought to environmental sites. This stands in sharp relief to traditional methods where samples are taken in the field and brought to a central (usually distant and costly) laboratory. The delays and costs of the old approach are considered increasingly unworkable. The only restraint in a full and complete conversion to field analyses today is the poor performance and limited capabilities with field instruments and resultant compromises in quality of analyses. This research program is in the mainstream of field instrumentation and could or should provide an highly portable field analyzer with advanced features not found on portable gas chromatographs (GC). Moreover, with attractions in size, weight, and power features, potential for true applications should be far better than those for fieldable mass spectrometers. The creation of the Volatile Organic Analyzer (VOA) for the International Space Station represents an instance where NASA has been pivotal in pushing technology beyond a state-of-the-art status and where instrumentation now exists where no such instrumentation had previously existed. Although the principles had existed before VOA and prototype instrumentation had existed, VOA is a technological milestone in the development of GC/IMS and field portable instrumentation.

Commercial implications are strong with applications in hazardous waste screening and industrial monitoring. The effects on ordinary citizens will be largely hidden though not inconsequential and will be linked to the

ultimate application of portable sophisticated analytical instrumentation. A clean environment, afforded through proper control and regulation of wastes, is the ultimate and proper application for terrestrial applications of these advances. Other applications may include monitoring of air and water supplies in a variety of scenarios including ventilation systems, water treatment facilities, waste steam lines (local or system-wide), and other industrial applications such as solvent and waste storage facilities. All of these have been predicated upon the availability of qualified, affordable instrumentation in an appropriate time-frame and the creation of VOA has set a standard in these endeavors.

FY97 Publications, Presentations, and Other Accomplishments:

Eiceman, G.A. "Advances in interpretation and prediction of ion mobility spectra: Future utilization of VOA." 27th International Conference of Environmental Systems, Lake Tahoe, NV (July 14 - 17, 1997).

Eiceman, G.A. "Thermal instability of chloride adducts of phenols." 6th International Workshop on Ion Mobility Spectrometry, Bastei, Germany (August 10 - 14, 1997).

Eiceman, G.A. and Stone, J.A. "Molecular origins of ion mobility spectra: A first step toward interpretative and predictive capabilities." Field Analytical Methods for Hazardous Wastes and Toxic Chemicals, Las Vegas, NV (January 28 - 31, 1997).

Ewing, R.G. Kinetics of gas phase decomposition of proton bound dimers of alkylamines. (Dissertation) New Mexico State University, Las Cruces, NM (1996).

Plasma Chemical Approaches to the Development of Biofilm-Resistant Surfaces

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Funding:

UPN/Project Identification: 199-04-12-01

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$132,000

Responsible NASA Center: Ames Research Center

Task Description:

This research is concerned with applying the techniques of low-temperature plasma polymerization and plasma surface modification of polymers to the development of antibacterial or biofilm-resistant coatings or surfaces on plastics, metals, or other substrata that could be used in a variety of aerospace, biomedical, or commercial applications. For NASA's Advanced Life Support programs, a major application would be conferring lasting biofilm resistance upon the piping or container walls in the closed-loop water reclamation system (WRS) for future space habitats. Biofilms on the surfaces of the WRS could harbor potentially pathogenic bacteria in the recycled water coming from showering, clothes laundering, or dishwashing and thereby present a hazard to astronauts on long-duration space missions as in the future International Space Station. Other applications which could emerge as NASA technology transfer to the commercial sector are antibacterial coatings in domestic or industrial systems involving humid environments (e.g., air conditioning units) or in the biomedical field (e.g., urinary catheters and intravascular devices). Since biofilms, once established, resist physical cleaning and penetration by biocides, their formation must be avoided or suppressed. This research aims to follow up unpublished results at Ames Research Center (ARC) which indicated that a coating (designated here as PPOM, and prepared by the plasma polymerization of a certain organic monomer, OM), imparted biofilm resistance to polyethylene, glass, and other substrata when exposed to a pure *Pseudomonas aeruginosa* culture. Accordingly, this research has as a major goal the study of the biofilm resistance of a family of coatings derived from the plasma polymerization of various organic monomers structurally related to OM when those coatings are exposed not only to *P. aeruginosa* but to other common pathogenic bacteria as well. The working hypothesis is that there is a particular chemical functionality (or functionalities) within the complex PPOM structure responsible for the observed antibacterial effect, and this notion needs to be tested by determining the relative biofilm resistance of a series of PPOM-like coatings containing varying amounts of the putative functionalities. At the same time, there is much scientific interest in determining the relative biofilm resistance of a homologous series of commercial plastics that are the conventional polymer analogues of the PPOM-like plasma polymers. Studies of both classes of polymers should lead to important structure-property relationships, something that has been lacking in the considerable literature on bacterial attachment to assorted polymers.

Research during the third and final year of this NRA grant continued to be concerned with plasma chemical approaches to biofilm-resistant coatings or surfaces. The work involved the joint efforts of a polymer chemist

and a plasma chemist at ARC to prepare and characterize a series of plasma-deposited coatings for later, extramural assessment of their resistance to biofilm formation when subjected to pure *Pseudomonas aeruginosa* culture. Because of disappointing biofilm-test results obtained during the first year at Southwest Texas State University, a new collaboration was established with the Center for Biofilm Engineering, Montana State University (MSU), which commenced in June 1996. Because of a slow start-up in biofilm testing at MSU and the fact that Dr. Bryers, the microbiologist responsible for that testing, left MSU in June 1997 with no plans to continue that work, progress on the biofilm testing of samples prepared at Ames did not advance much beyond what was reported in the FY96 Task Progress Report, submitted at the end of February 1997. Before discussing the limited accomplishments subsequent to that report, and for purposes of completeness, it is useful to briefly summarize the prior work.

Four separate batches each of 30 coated (with plasma-polymerized tetrafluoroethylene or PPF4) and 30 uncoated (control) polyethylene (PE) samples were supplied to Dr. Bryers for full biofilm testing against *P. aeruginosa*. Two of the coated batches (BF1 and BF2) had the same thickness of PPF4 deposit (ca. 115 nm), while the other two coated batches (BF3 and BF4) had different thicknesses (ca. 58 and 230 nm, respectively). BF1 was used at MSU to develop methodology and reproducibility, while the other three batches were used to (a) quantify the kinetics and extent of adhesion of *P. aeruginosa*, and (b) determine if thickness of coating had an effect on that adhesion. Biofilm testing at MSU comprised determination of total cell numbers, total biofilm protein, and total biofilm polysaccharide per area on the coated and uncoated samples as a function of time of exposure to *P. aeruginosa*. That testing was to include FT-IR assessment of biofilm formation, but due to an FT-IR instrument failure at MSU, most of the biofilm-coated samples had to be returned to ARC for infrared analysis, using the approach developed several years ago at ARC. Generally, but not consistently, bacterial exposure of coated and uncoated PE samples yielded S-shaped kinetic plots for cell counts, polysaccharide and protein contents, as well as extent of biofilm formation as measured by FT-IR. Surprisingly, the biofilm uptake in the coated samples—as indicated by the four measurements—usually exceeded that of the uncoated samples, and deposit thickness had no apparent effect on bacterial adhesion, both findings contrary to expectations based on early research at ARC. The results of these tests led to the conclusion that, insofar as *P. aeruginosa* culture was concerned, PPF4 coatings showed no promise in leading to the desired antibacterial coatings. Plans to examine biofilm testing of plasma-polymerized coatings of homologues of PPF4, namely PPF1, PPF2, and PPF3 (based on vinyl fluoride, vinylidene fluoride, and trifluoroethylene, respectively) had to be abandoned with Dr. Bryers' termination of experimental work on this project. However, he is currently preparing a draft manuscript for potential publication describing the experiments performed at MSU, which will incorporate FT-IR data obtained at ARC. The transmission FT-IR technique employed is novel and worthy of publication in its own right, and allows for semi-quantitative assessment of entrained water, proteins, and polysaccharides as well as indications of non-uniformity of biofilm layering.

Following Dr. Bryers' relocation from MSU, there was too little time left in the third year of this NRA project to seek another microbiologist to perform biofilm testing on plasma-polymerized coatings. As a result, the emphasis on research at ARC was redirected towards alternative ways of depositing fluoropolymer surfaces on various substrates. Although biofilm-resistant coatings based on PPF4 were not forthcoming, the possibility of achieving antibacterial surfaces through plasma deposition of polymeric films remains real, as evidenced by recent work [E.E. Johnson, J.D. Bryers and B.D. Ratner, *Polymer Preprints*, 38 (1), 1016 (1997)] on exposure of plasma-deposited polyethylene oxide-like thin films to *P. aeruginosa*.

Two methods for deposition of polymer coatings that have been pursued involve rf plasma sputtering of polytetrafluoroethylene (PTFE) with an inert gas (e.g., argon) or sputtering of PTFE with an argon ion beam. On the former method, the FT-IR, UV, and XPS spectra of plasma-polymerized tetrafluoroethylene (PPTFE) and of the fluoropolymer deposits (SPTFE) formed from rf plasma sputtering of PTFE using He or Ar as sputtering gas were compared. This study was the first to involve preparation of PPTFE and SPTFE in the same reactor and under comparable low-power plasma conditions. Literature suggestions of a similarity in PPTFE and SPTFE structures, based on IR or XPS spectra, were confirmed, with some differences noted. A claim questioning that similarity based on UV spectra for SPTFE and plasma-polymerized carbon tetrafluoride was shown to be erroneous. The C1s XPS spectra of SPTFE formed using He or Ar (SPTFE-H or -A) displayed

relatively higher contents of CF₂ groups, and yielded higher F/C ratios, than PPTFE. PPTFE, besides having a lower fluorine content than SPTFE, also had a greater degree of conjugated unsaturation. A manuscript describing these results is about to be submitted for publication.

On the second method for polymer deposition, IR, UV, and XPS analyses have been carried out on three sets of fluoropolymer films resulting from argon ion sputtering of PTFE carried out at NASA-Lewis Research Center. These sets of films were produced under the same power and ion beam current but for various periods of time, and they had correspondingly varied thicknesses. The immediate interest was to compare the structures of the target PTFE and the resulting SPTFE deposits as a function of thickness. While there has been some literature on argon ion-sputtered PTFE (i.e., on the surface modification of PTFE subjected to argon ions), there has been scarcely any reports on the nature of the SPTFE deposits arising from the argon ion bombardment. New spectroscopic data have been obtained which provide added information concerning the chemistry of SPTFE beyond the limited XPS data reported a number of years ago [T. Wydeven, M.A. Golub and N.R. Lerner, *J. Appl. Polym. Sci.*, 37, 3343 (1989)]. This new material is being prepared for publication.

As a final note, sputtering, whether by rf plasma or inert gas ions, can be an efficient method for producing coatings with structures closely comparable to those of the target polymers. From the point of view of developing biofilm-resistant coatings, it would be of interest to examine sputter-deposited coatings derived from polyethylene oxide (PEO) as an alternative to rf plasma polymerization of PEO-like films.

Bacterial cells attach to almost any surface in contact with an aqueous medium. Once attached, the cells grow, reproduce and produce extracellular polymeric substances (predominantly exopolysaccharides) which provide a matrix for a community of trapped, living microorganisms known as a biofilm or microbial film. Biofilms possess either beneficial or undesirable properties depending upon their involvement. Since this research is aimed at biofilm-resistant surfaces, the present discussion of potential Earth benefits is limited to situations involving the undesirable properties of biofilms. An example of such a situation is the costly biofouling of ship exteriors, water pipes, heat exchangers, and various industrial engineering systems promoted by bacterial attachment to all kinds of surfaces - metal, ceramic, plastic, or glass. Another example, in domestic or industrial systems involving humid environments, is undetected biofilm formation in air conditioning units which, under rare and very adverse conditions, could provoke an episode of Legionnaires' disease. Likewise, biofilm formation in the air-circulation ducts of commercial aircraft can expose passengers to potential health problems, while the case of potential biofilm formation in the water reclamation system(s) of long-duration space missions (such as the future International Space Station) has been noted above. In the medical area, nosocomial infections arising from unrecognized biofilm formed on the surfaces of catheters and intravascular devices—infections that often result in fatalities—are quite common. Indeed, biofilm-layered, urinary catheters and attendant urinary tract infections are the major cause of morbidity in hospitalized patients. Other biomedical examples where biofilms can play an unpleasant role are contact lenses and various artificial prosthetic devices. Thus, there are many Earth benefits to be derived from developing biofilm-resistant surfaces or coatings. It is worth stressing that biofilms, once established, resist physical cleaning and penetration by biocides, and their formation must therefore be avoided or suppressed.

FY97 Publications, Presentations, and Other Accomplishments:

Golub, M.A. and Wydeven, T. "Relative rates for plasma homo- and copolymerizations of olefins in a homologous series of fluorinated ethylenes." National Meeting of the American Chemical Society, San Francisco, CA. *Polymer Preprints*, 38 (1), 1035 (April 13 - 19, 1997).

Golub, M.A., Wydeven, T., and Johnson, A.L. "On the similarity of plasma-polymerized tetrafluoroethylene and RF plasma-sputtered polytetrafluoroethylene." National Meeting of the American Chemical Society, Las Vegas, NV. *Polymer Preprints*, 38 (2), 668 (September 7 - 12, 1997).

In-site, Remote Chemical Sensors Based on Thin Magnetic Films

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Funding:

UPN/Project Identification: 199-04-17-25

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$128,748

Task Description:

This proposal seeks funding for the development of a new type of sensor that offers the opportunity for remote, *in situ*, continuous, long-term monitoring of given chemical stimuli. The sensor is comprised of a thin polymeric layer made so that it swells in the presence of certain stimuli, bounded on each side by a magnetically soft thin film. If the polymeric layer is thin, the magnetic switching characteristics of the sensor 'sandwich' is a function of the thickness of the intervening polymeric spacer layer. Placed within a low frequency, sinusoidal magnetic field, the magnetization vector of the sensor periodically reverses directions, generating magnetic flux that can be detected as a series of voltage spikes in suitably located detecting coils. The general shape and magnitude of the generated voltage spikes are dependent upon how much the spacer layer has swollen in response to the given stimuli. The polymeric chemistry is very flexible, and can be made to selectively swell in response to, for example, glucose, moisture, pH, ion concentration, and the presence of trace (including heavy) elements; in response to NASA's needs this proposal primarily addresses atmospheric CO₂ measurement.

The sensor principle has been demonstrated using lightly crosslinked poly (dimethylamino) ethylmethacrylate to detect pH. The interrogation and detection electronics are based upon magnetic identification marker systems widely used, for example, in libraries and CD shops, where sensors are detected over a range of several meters. Since no physical connections such as wires or cables are needed to obtain sensor information, nor line of sight as needed with laser diodes, they are ideally suited for placement within structures such as airways, storage containers, spacesuits, etc. The ultimate size of the sensor is a function of magnetic material properties and size of the observation area; current magnetic identification markers that can be readily monitored over several meters are approximately 1 μm thick with an area of $\approx 6 \text{ cm}^2$. Since there is nothing to wear out, the sensor can last indefinitely unless it is physically destroyed.

The primary research objectives of this proposed work are: (1) Refinement of the thin film polystyrene (aminated with diethanolamine) fabrication process, covalently bonding the required thin magnetic films to each side of the polymer. This material is sensitive to pH, swelling due to charge-charge repulsion when the amine group is protonated by exposure to acid; (2) Extend the polymer chemistry to swell in the presence of CO₂; (3) Determine optimal sensor fabrication processes for high performance, easily detected sensors; and (4) Extend current sensor detection schemes for increased area coverage and enhanced sensor monitoring.

Although the proposed technology is new and quite exciting, its fundamental principles are based upon well proven and demonstrated technologies; therefore, there appears to be little inherent risk in the sensor development.

Earlier magnetochemical designs suffered from slow response times, which were on the order of days, since the chemical analyte had to diffuse in from the edge of the sensor. Since the polymer edge was a few microns in thickness, and the length of the sensor approximately a centimeter, response times were slow and the unequal stress distributions led to premature failure of the sensor via delamination between the polymer and magnetic film.

We have made a considerable leap in our technology by fabricating thin film sensors comprised of a polymer layer, approximately 300 microns in thickness, upon which a thin film magnetic geometry is attached. Our greatest magnetic response is found with a square-like magnetic thin film structure (23 microns thick), in which the square has been divided into four triangles by cutting the thin film along the two diagonals. As the polymer swells and shrinks, the spacing between the triangles changes, resulting in changes in the magnetostatic coupling between the triangles. This dramatically changes the overall magnetic response of the sensor. We have tested several such thin film sensors, and find that the sensors respond within a matter of minutes to the analyte, and to date have been cycled between high/low pH solutions approximately 30 times without delamination problems.

Work is continuing on development of a physically robust polymer that will respond to changes in pH in a matter of seconds, and optimization of the magnetic design and fabrication details.

This research seeks to develop a new type of sensor that will be suitable for many applications, including space and ground-based medical monitoring. The sensor is unique in that sensor information is obtained by changes in magnetic flux, which can be monitored remotely without any connecting wires or line of site telemetry. The chemistry and applicability of the sensor is great; in one variation the sensor could be used to monitor *in situ* glucose levels, atmospheric CO₂ levels for both environmental monitoring, and monitoring of food quality in sealed packages, etc.

Rapid Bacterial Testing for Spacecraft Water

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Co-Investigators:

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Funding:

UPN/Project Identification: 199-04-17-18
Initial Funding Date: 1996
Students Funded Under Research: 2
FY 1997 Funding: \$98,486

Solicitation: 95-OLMSA-01
Expiration: 1998
Post-Doctoral Associates: 1

Task Description:

In water microbiology, there is a need for rapid methods to enumerate specific viable bacteria. This is a particular concern in relation to water which will be reclaimed for potable use on the International Space Station. Our principal objective is to develop procedures which will permit the detection of specific marker bacteria which would be used to monitor the performance of the water reclamation and storage systems. In addition, the techniques will be applicable to the detection of particular pathogenic bacteria. Our novel approach (patent pending), which utilizes membrane filtration and combines a fluorochrome for assessment of respiratory activity with specific fluorescent antibody detection of waterborne bacteria, will be evaluated in comparison with molecular methods which will be developed in this project. These include fluorescent *in situ* hybridization following membrane filtration and microcolony formation to permit rapid quantitation of specific, viable bacteria. Fluorescent *in situ* PCR will also be investigated for sensitive detection of specific bacteria. Results will be applicable not only to spacecraft systems but will also have applications for Earth-based situations. Similar methodologies would be of great value for the examination of clinical and fecal specimens, potable waters, natural waters, foods, and soils, for more timely and reliable detection of specific microbial contamination. Other applications include the examination of purified waters used in the pharmaceutical industry, laboratories, and the electronics industry.

Experiments were performed using a suite of fluorescent stains that demonstrate a range of cellular physiological activities and properties that are related to viability. These stains were applied to the pathogen *Escherichia coli* O157:H7 exposed to low levels of disinfection with chlorine. Freshly prepared suspensions and starved cultures were examined. With earlier funding, we developed a rapid method for detecting specific respiring bacteria. A prototype system has been demonstrated with *Escherichia coli* O157:H7 suspensions and inoculated ground beef. In FY97, we have continued evaluation of this hybrid technique, which includes immunomagnetic separation and incubation with cyanoditolyl tetrazolium chloride (CTC) to determine respiratory activity, followed by fluorescent antibody staining (IMS/CTC/FAB).

Our objective is to develop analytical procedures to identify and quantify bacteria in waste water and product water on spacecraft, permitting more timely measurement and control of bacterial contaminants, and facilitating development of standards and countermeasures to optimize crew health, safety, and productivity.

We have used a suite of stains and probes, in conjunction with viable plate counts, to assess the effect of chlorine disinfection on membrane potential (Rhodamine 123 [Rh123] and DiBAC₄3), membrane integrity (BacLight Live/Dead Kit, Molecular Probes Inc.), respiratory activity (CTC), and substrate responsiveness (direct viable count [DVC]) in *E. coli* O157:H7. After a 5 minute exposure to chlorine, physiological indices were affected in the following order: viable plate count > substrate responsiveness > membrane potential > respiratory activity > membrane integrity. *In situ* assessment of physiological activity using a multi-phasic approach, as demonstrated in this study, permits more comprehensive decisions to be made in regard to determining the site and extent of injury in bacterial cells.

In related experiments, cultures of *E. coli* O157:H7 were starved in M9 minimal medium with no added carbon source, for 14 days at 21-23°C. In addition to the assays listed above, intracellular esterase activity was determined using Fluorasure (Chemunex, France) in which case bacteria on filters were enumerated with a Scan RDI (Chemunex) rapid laser scanner. Assays were performed to demonstrate the influence of starvation on susceptibility to disinfection with 0.5 ppm chlorine. Results indicated that between inoculation and starvation through day 5, the assays used demonstrated an increased number of physiologically active cells, with numbers remaining relatively constant through day 14. However, cells exhibiting membrane potential and substrate responsiveness declined as starvation progressed, while resistance to chlorine disinfection increased as indicated by the percentage of injured cells. Injury decreased from 92.6% of cells at time 0, to 44.3% by day 5, and remained relatively constant through day 14 (35.3%). The results show that in the starvation conditions used, this strain of *E. coli* maintained physiological activity while increasing resistance to chlorination.

For the IMS/CTC/Fab evaluation, we have added the more rapid and sensitive Scan RDI system. Using the IMS/CTC/Fab procedure, we recovered more than 86% of the O157 cells in the inoculum, with regression coefficients > 0.95 when comparing the CTC/Fab counts with those obtained by plate count enumeration of cells recovered by immunomagnetic beads. Epifluorescence microscopy has a lower detection limit of ca. 10³ cells per g or per ml of sample, while the Scan RDI system permits detection of 1 cell per g or per ml.

The results of experiments conducted in this period demonstrate the utility and versatility of the novel rapid analytical methodology under investigation. Although only one of the present suite of physiological fluorochromes as well as the IMS/CTC/Fab were compatible with the current Scan RDI instrumentation, our results thus far provide justification for optimism concerning the prospect of applying this powerful new rapid approach to bacterial detection in a wide range of settings. Discussions with the manufacturer of the Scan RDI system continue to focus on avenues for improving the versatility and cost of this instrument as it is reconfigured. In addition, we will continue to evaluate and validate additional physiological fluorochromes for this kind of instrumentation, based on our experience with stains that assess specific physiological targets. Ultimately, this line of investigation will lead to more accurate methods for bacterial identification and viability determination associated with this rapid detection strategy.

The techniques we are developing for rapid detection of specific bacteria in conjunction with viability assessment have attracted significant attention among environmental microbiologists. The combination of immunomagnetic separation with the CTC respiration assay and fluorescent antibody staining permits direct detection of bacterial contaminants within six-seven hours. Most other methods employ a 12-24 hour enrichment prior to identification. A patent application on this technology is currently being evaluated. One of the impediments to timely assessment of water and food quality has been the time required to obtain results using traditional or even novel techniques. These procedures will be used for monitoring potable water, foods, and parenteral (injectible) liquids.

Our experiments with disinfection and starvation, using these new methods to detect bacteria, will generate more reliable data on bacterial injury, lethality, and survival. The techniques we are developing for rapid detection of specific bacteria in conjunction with viability assessment have attracted significant attention among environmental microbiologists. The combination of immunomagnetic separation with the CTC respiration assay and fluorescent antibody staining permits direct detection of bacterial contaminants within six-seven hours. Most other methods employ a 12-24 hour enrichment prior to identification. A patent application on this

technology is currently being evaluated. One of the impediments to timely assessment of water and food quality has been the time required to obtain results using traditional or even novel techniques. These procedures will be used for monitoring potable water, foods, and parenteral (injectible) liquids. Our experiments with disinfection and starvation, using these new methods to detect bacteria, will generate more reliable data on bacterial injury, lethality, and survival. The combination of methodological approaches for bacterial concentration, detection and viability assessment being investigated has significant benefits both immediately and in the long term. For example, our present target bacterium (*E. coli* O157:H7) is currently a persistent and significant food borne health threat in the U.S. Other aspects of developing this technology have been funded by the U.S. Department of Defense (Army) and the National Institutes of Health (Environmental Health Institute). A patent is pending, and we have partnered with Montana ImmunTech, Inc., a local biotechnology development company to bring this technique to the U.S. and international market place. Ultimately, the development of novel antibodies may lead to therapeutics and possibly vaccines for the treatment of a range of enteric diseases which are caused by bacteria.

It is anticipated that our new methodological approach will be applied in studies by others to determine the ecology of *E. coli* O157 and related bacteria in the food source animal population and their environment and, as a consequence, assist in its reduction or elimination. Some version of this technology might also be amenable to miniaturization and use in protecting crew health on space vehicles. It is also applicable to the rapid detection of other target bacteria in a range of contexts including industrial, clinical, and environmental settings.

FY97 Publications, Presentations, and Other Accomplishments:

Lisle, J.T., Pyle, B.H., and McFeters, G.A. "Evaluation of physiological activity in *E. coli* O157:H7 after disinfection." American Society for Microbiology, 97th General Meeting, Miami Beach, FL, Abstracts, p. 455, Q-3 (May 4 - 8, 1997).

Pyle, B.H., Broadaway, S.C., and McFeters, G.A. "Rapid enumeration of respiring *Escherichia coli* O157:H7 in water and meat using immunomagnetic separation." American Society for Microbiology, 97th General Meeting, Miami Beach, FL, Abstracts, p. 480-480, Q-151 (May 4 - 8, 1997).

Pyle, B.H., Lisle J.T., Broadaway, S.C., and McFeters, G.A. "Rapid detection of bacteria in spacecraft water systems." SAE Technical Paper Series No. 972421. 27th International Conference on Environmental Systems, Society for Automotive Engineers, Lake Tahoe, NV (July 14 - 17, 1997).

Miniaturized Liquid Chromatography

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Funding:

UPN/Project Identification: 199-04-17-17

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$126,858

Task Description:

This research program focuses on the development, testing, and implementation of novel chemical sensor instrumentation for operation in long-term, closed-loop, space-life support systems. Of particular need is instrumentation for the detection, identification, and quantitation of organic contaminants in water generated by on-board recycling/management systems. Such an instrument should also function in an alarm mode in the event of contamination. To this end, we are developing a new class of analytical instrumentation. Our strategy couples the well-known capabilities of liquid chromatographic (LC) and flow injection analysis (FIA) methods with opportunities for extremes in miniaturization using micromachining techniques to integrate all of the key functional components (i.e., column, detector, pump, and injector) into a chip-scale instrumental platform. Central to our effort is the creation of a chip-scale, low voltage pump that can also function as a sample injector for the controlled delivery and metering of fluids. Importantly, the development of a low voltage pumping system addresses a critical need in the eventual realization of "chemical analysis on a chip." As such, the new instrument will represent an extensive size reduction for payload minimization and greatly reduce the consumption of materials as well as the generation of wastes.

Pump Design. Efforts at ISU this past year have been focused largely on the design, construction, and testing of our electrochemically activated micropump. As shown in proof-of-concept designs, a pool of mercury contacts two cylindrically-shaped, concentrically-aligned fluid reservoirs, with the outer reservoir filled with an aqueous electrolyte and the inner reservoir contacting the sample solution. A set of ball-style check valves, which were manufactured to our specifications by Ace Glass, Inc., are incorporated into the pump configuration for achieving one-directional flow. Platinum wires are inserted into both the mercury pool and the aqueous electrolyte for electrical connection to an external voltage source. In the absence of an externally-applied voltage difference across the two contacts, the equilibrium heights of mercury in each reservoir reflect a balance of interfacial and gravitational forces. Importantly, electrochemically-induced changes in the surface tension ($\Delta\gamma$) of the mercury in the outer reservoir of the pump result in a change in the radius of curvature at the electrolyte-mercury interface in the outer reservoir. The force generated by this change in shape subsequently drives a displacement of the mercury in the inner reservoir. Thus, the movement of the column of mercury within the inner reservoir acts as a piston-like pump, with the extent of displacement proportional to the amplitude of the voltage difference and the oscillation frequency controlled by the frequency of the applied voltage waveform.

To delineate performance characteristics, we first modeled the accessible upper limit of the pressure generated by our pump for pushing liquids through small bore tubing. The fundamental relationships governing the correlations of $\Delta\gamma$ with the displacement in the height of mercury in the inner reservoir (Δh), the pressure generated by $\Delta\gamma$ (ΔP), the volume flow rate (F), and the pressure gradient ($\Delta P/L$) are:

$$(1) \quad \Delta h = 2 \Delta\gamma / \rho g$$

$$(2) \quad \Delta P = 2\Delta\gamma / r$$

$$(3) \quad F = \pi r^4 / 8\eta_m \Delta P / L$$

where L is the flow channel length, r is the internal radius of both the inner reservoir and the flow channel, ρ is the density of mercury, g is the gravitational acceleration, and η_m is the viscosity of the pumped liquid. With the voltage window accessible for aqueous solutions in contact with mercury, the maximum value of $\Delta\gamma$ is ~ 100 dyne/cm. Using the above equations, we can estimate values of ΔP and $\Delta P/L$. For example, at column and inner reservoir radii of 500 μm , the value of ΔP generated by the pump is 0.06 psi. If then we use a column length of 10 cm and flow rate of 1000 nL/min, the corresponding pressure drop using water as the sample solution is 1.0×10^{-5} psi. These estimations argue that our pump has the potential to provide operational pressures that are more than adequate to drive aqueous solutions at flow rates (e.g., a few microliters per minute) and column lengths (e.g., 10-50 cm) of utility for miniaturized FIA and LC.

Pump Performance. The flow rate capability is one of the most important delimiters in defining pump performance. We acquired such data by measuring the flow volume per unit time and by metering the displacement length of the fluid per stroke (i.e., an individual displacement cycle) at low pumping frequencies.

Volume flows per stroke as small as 0.5 μL and up to ~ 12 μL were observed, confirming the ability of our actuation concept to function effectively as a fluid pumping system.

Our preliminary tests also demonstrate a second critical attribute of our pumping system - low power consumption. We have found that the maximal current drawn by our pump at its present size is at most only a few milliamperes, a level that reflects the current flow to charge of the electrical double layer formed by the contact between mercury and the supporting electrolyte. These levels of current, coupled with a 2-volt upper limit in the differential voltage that can be applied at mercury in contact with aqueous solutions, translate to a peak power consumption of a few tens of milliwatts. We note that these power levels are readily accessed from the small-sized power cells used to operate modern conveniences such as wrist watches and cameras.

Flow Injection Analysis. To demonstrate applicability to fluid delivery, the pump was incorporated into a miniaturized FIA system that was assembled from glass or plastic capillary tubing and machined plastic platforms. The mercury pump was connected to two ball-type check valves to achieve one-directional fluid flow, and to an electronically-controlled, three-way valve to select between sample and carrier streams.

An aqueous solution of 2 mM ferrocynide was used as the carrier, and different volumes of a 5 mM aqueous cerium(IV) solution was injected to the carrier stream. Mixing is accomplished by the diffusion of the sample zone and the carrier stream. Cerium(IV) is therefore reduced to cerium(III) and ferrocynide is oxidized to ferricyanide, with the progress of the redox reaction monitored by the absorbance of ferricyanide at 430 nm. The experimental results show that the absorbance increases linearly as the injection volume of cerium(IV) increases. We also note that the tailing of the bands for sample elution follow the dispersional dependence expected for the differences in sample volume.

Other types of FIA experiments have been examined using the single-line setup. For example, we have used this setup to conduct an acid-base titration by using the absorbance data collected upon injecting different volumes of a NaOH solution into a HCl/methyl red carrier stream. We have also demonstrated the analysis of a

complexation reaction between Fe^{3+} and SCN^- , as well as conducted preliminary analyses based on electrochemical detection. Taken together, these results provide a strong foundation for the continued development of our novel pumping system.

Efforts during the next year will continue on the noted optimization of the performance of our electrochemically activated pump. Efforts will expand to the evaluation of the performance of the silicon-based flow channels for use in both FIA and LC. These studies will entail the development of correlations between the diameter of the flow channel, back pressure, and flow rate. These tests are aimed at more clearly defining the pressure requirements needed to meter fluids accurately at very low flow rates, i.e., a few nanoliters per minute. These evaluations will be conducted by determining gravimetrically the total volume flow and/or by placement of a microelectrode inside the exit of the column. In the latter case, the current that flows from the redox reaction of a couple like ferri-ferrocyanide will be used to monitor the consistency of the flow rate.

Investigations will also involve the development and/or refinement of analytical procedures for both of our miniaturized LC and FIA systems. In the case of the former, we will explore the modification of the walls of the silicon-based columns using, for example, silicone coupling agents. This approach will be employed for the creation of a LC system that operates in a reversed-phase mode. Tests will include characterizations of the extent and stability of the wall modifications and the efficiencies of the separations of shortchain hydrocarbons and alcohols. Studies of FIA will parallel those of LC, focusing primarily on the efficiency of the mixing of the sample and carrier streams.

The JPL portion of the project has focused in Year I on two related efforts: column construction and check valves for one-directional fluid flow.

Column Construction. Several processing techniques were investigated and characterized for fabricating in silicon the microcolumns for use in our miniaturized LC and FIA systems. These included the isotropic wet chemical etching of pyrex wafers, the mechanical drilling of pyrex wafers, the isotropic wet chemical etching of silicon wafers, anodic bonding of silicon wafers to pyrex wafers, and the photolithographic definition and structural resolution of micro-columns. Based on these efforts, prototype columns were fabricated with isotropically etched channels on a silicon wafer, a pyrex wafer with ultrasonically drilled access holes. These two components were aligned and sealed together by anodic bonding. This platform has recently been delivered to ISU for flow tests, which are underway.

Check Valve Construction. Our preliminary investigations of microfabricated one-way check valves have focused on designs that are suitable for our two analyzer systems. The first conceptual approach is based on a combined bulk and surface micromachining technique. The design includes concentric annular corrugations that can be incorporated to improve sealing and to trap microparticles, a thin sacrificial layer that can be used to define precisely the leaf-to-seat alignment, and a conformal thin-film deposition that can be used to form the valve leaf to fit over the valve seat. Operationally, we envision that the valve is kept in a normally closed state with four pre-biased springs, which are fabricated with a stack of two thin films with different post-process built-in stress to achieve a bimetallic actuation process.

Efforts in the next year will continue the design and construction of the flow channels to enhance flow characteristics as judged from the ongoing evaluation of the first generation platform. We will also begin the fabrication of a new check valve design, and initiate the design of the a micropump.

We note that J. Ni, a graduate student in chemistry at ISU, will be working at JPL for at least two months in the upcoming year at the microfabrication facilities of JPL as part of the cross-disciplinary research and education of the overall program.

Conventional sampling and analysis techniques often incur substantial labor and equipment costs. Because the number of chemicals falling under drinking water regulations continues to increase, costs will continue to escalate unless new monitoring technologies that operate in a real-time, low-maintenance mode are developed.

The proposed chemical analysis instrumentation which can be used in a liquid chromatographic and/or flow injection analysis mode potentially targets this critical need.

To estimate roughly the potential impact of the proposed instrument, it is instructive to assess the costs of conventional sampling and analysis techniques. Although only a beginning, the development of a field-deployable instrument offers a potentially dramatic reduction in monitoring costs. The needs of the water treatment industry for disinfection by chlorine will serve as an example. Currently, the expense of manual testing derives mostly from labor costs. Based on costs incurred by conventional testing laboratories, each manual test conservatively costs ~\$3.30. Automatic analyzers, while more expensive than test kits, run ~\$2.45 per day. It is projected that the reduction in associated costs using the proposed technology would lead to a 25% reduction in analysis costs when compared to existing automatic analyzers, and a 45% reduction in analysis cost over manual testing.

Even more important to the cost impact of miniaturization is the reduction in volume of spent reagents, samples, and other chemicals for waste handling. Chemical waste disposal costs vary widely depending on the nature of the waste and local disposal regulations. The increasing cost for disposal of chemical waste underscores the need for the proposed analytical systems, which would reduce waste generation. Given the remote location of sampling points, for example, in the above disinfection analyses, the waste generated by an automatic analyzer generally becomes a point-source discharge. A typical batch-flow analyzer, generating 100 milliliters of waste per determination, would produce 20,000 liters of waste per year. This output translates into an estimated disposal cost of ~\$10,000 per year, assuming the waste stream is devoid of regulated materials. The cost of disposal could easily exceed \$39,000 per year for regulated materials (e.g., heavy metals). Our proposed technology, operating, for example, at fluid flows of five microliters per minute, would generate three liters of waste per year and require 66 years to fill a 200 liter drum. Disposal costs would be between only \$62 to \$66 per year.

The above analysis reveals that the proposed instrumentation has the potential to have a major impact on environmental monitoring, a critical Earth-bound need. This technology also has applications to other important areas, including the on-line monitoring and control of chemical industrial processes. As with environmental monitoring, the majority of process control determinations are conducted in laboratories removed from the sampling points. This situation can incur additional manufacturing costs because of incorrect product formulations as well as from the generation of additional waste.

FY97 Publications, Presentations, and Other Accomplishments:

Coldiron, S.J., Zhong, C.-J., and Ni, J. "The multistat: A computer-controlled multi-potentiostat for the programmed actuation of electrochemically-driven micropumps in a peristaltic-type array configuration." Pittsburgh Conference, Atlanta, GA (March, 1997).

Ni, J., Zhong, C.-J., and Coldiron, S.J. "Miniaturized flow injection analysis with an integrated electrochemically-driven micropump." Pittsburgh Conference, Atlanta, GA (March, 1997).

Porter, M.D., Zhong, C.-J., Ni, J., Coldiron, S.J., and Tang, W.C. "Electrochemically-driven micropump for fluid flow and delivery: Toward a novel micropump design for miniaturized liquid chromatographic and flow injection analyses." 27th International Conference on Environmental Systems, SAE Technical Paper Series, No. 972420.

Zhong, C.-J., Ni, J., and Coldiron, S.J. "Electrochemically-driven micropump for fluid flow and delivery: Interfacial double-layer charging/discharging and performance correlations of a novel micropump design." Pittsburgh Conference, Atlanta, GA (March, 1997).

Zhong, C.-J., Ni, J., and Coldiron, S.J. "Mercury micropumps: A novel approach toward a miniaturized, low power fluid delivery system." Pittsburgh Conference, Atlanta, GA (March, 1997).

Pulse Tube Refrigeration New Techniques for Improving Efficiency

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

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Task Description:

The work proposed here consists of three phases, each of one year in length. Phase I consisted of evaluating tapered and stepped regenerator geometries as a means of improving the efficiency of the pulse tube refrigerator. First, the effects of tapering and steps were evaluated using NIST REGEN3.1 software as well as a thermoacoustic code. Both codes showed very little (<10%) improvement in the efficiency. An apparatus to test different regenerators and pulse tubes was fabricated in this phase. For phase II, measurements were made of the thermal conductance through stacked screens to provide the data needed for the calculation of the optimum geometry. Measurements were made with stainless steel and phosphor bronze screen of several mesh sizes and different porosities. Several regenerators of different geometries were fabricated and tested in the test apparatus and compared with the calculations. Good agreement was found and a no-load temperature of 35°K was achieved. In phase III, we propose to investigate the effect of pulse tube geometry, including volume, aspect ratio, and taper. We also plan to develop a model of the inertance tube based on transmission line theory to compare with experimental values. Improvements to the overall efficiency due to the use of the inertance tube will be investigated.

Progress for FY97 included the following areas: (1) the effect of phase angle on regenerator losses; (2) a comparison of losses in pulse tube and Stirling refrigerators; (3) measurements of the axial thermal conductance in several packed screen regenerators; (4) calculation of optimum regenerator geometry with the new conduction factor; and (5) experimental tests with various regenerator geometries.

The NIST software REGEN3.1 was used to calculate regenerator losses (heat transfer and pressure drop) as a function of the phase angle between the mass flow and the pressure at the cold end of the regenerator. Entropy generation was used in the calculations. The total of the two losses showed a minimum at a phase angle of about -30 degrees (mass flow lagging pressure). Such phase angles can be obtained in Stirling refrigerators, but

typically a phase angle of about 0 degrees is the best in pulse tubes with a double inlet. The use of inertance tubes in large pulse tube refrigerators can provide larger phase shifts and allow for a -30 degree phase angle.

Both REGEN3.1 calculations and previous experimental measurements were used to evaluate all the losses in a pulse tube refrigerator. Compared with the total entropy generated within the low temperature components, about 40% originates from the regenerator, 35% from the pulse tube, and 25% from the net refrigeration power. Thus, a small reduction in regenerator or pulse tube losses can have a significant impact on increasing the net cooling power and the overall efficiency. Losses in the Stirling refrigerator are about 35% for the regenerator and 15% for the expansion space. Thus, reducing the pulse tube loss (possibly by the use of a tapered pulse tube to reduce flow streaming) as well as that in the regenerator can lead to significant improvements in the pulse tube refrigerator efficiency compared with that of the Stirling refrigerator.

Measurements of the axial thermal conductance through stacked screen regenerators were made with 400 mesh stainless steel screen of two different wire diameters, 325 mesh stainless steel screen, and 325 mesh phosphor bronze screen. By varying the force on the end plugs, the measurements were made with different porosities. The internal helium gas pressure was also varied from zero up to 2 MPa to allow for a determination of the heat transfer mechanism. These measurements showed that most of the heat is conducted across the boundaries by the helium gas within about 4 micrometers from the wires. The thermal conduction degradation factor associated with the many layers of screen was found to be about 0.1 for stainless steel screen and 0.022 for phosphor bronze screen. Previously a factor of 0.3 had been used in calculations of conduction loss for the stainless steel screen. A factor of 0.1 leads to an optimized regenerator that is shorter and larger diameter. A paper on these measurements was presented at the 1997 Cryogenic Engineering Conference and was accepted for publication in the proceedings.

Calculations of the optimum regenerator geometry were performed using REGEN3.1 with the new conductivity factor of 0.1 for stainless steel screen as well as with the old factor of 0.3. For experimental measurements, both stainless steel and phosphor bronze screens were used in the test regenerators. Regenerator tubes were made with stainless steel and Ti6Al4V titanium alloy. The lowest no-load temperature of 35°K was achieved with the regenerator that was optimized by the REGEN3.1 numerical model. This no-load temperature is the lowest ever reported for such a small system. That optimum regenerator was made with the titanium alloy filled with 500 mesh stainless steel screen. Its length was 32 mm and the diameter was 9.2 mm I.D. The pulse tube refrigerator was driven with a commercial compressor that had a swept volume of only 4 cc and operated at 54 Hz. A net refrigeration of 2.5 W was achieved at 80°K. A paper on these regenerator comparisons was presented at the 1997 Cryogenic Engineering Conference and was accepted for publication in the proceedings.

This research pertains to improved methods for cooling biological specimens in space. Earth benefits of the improved cooling include potential cooling of high temperature superconductors for use in cellular phone base stations to provide more channels and increased range, to make cellular phones available to more people, and to reduce interference in signals. The improvements in cooling techniques found here could be used in the liquefaction of natural gas for cleaner transportation fuel. These improved coolers can be used for cooling infrared sensors to study atmospheric phenomena such as the ozone hole and greenhouse effects. Use of these improved coolers by the Defense Department to cool infrared sensors would improve our surveillance capability. The improvements found in this program could also be incorporated in multistage coolers for temperatures down to about 15°K for use in improved cryopumps with less vibration for the semiconductor manufacturing industry. The reduced vibration allows for less defects in the fabricated chips and permits more compact packaging of the chips, resulting in higher speed operation.

FY97 Publications, Presentations, and Other Accomplishments:

Lewis, M.A., Kuriyama, T., Kuriyama, F., and Radebaugh, R. "Measurement of heat conduction through stacked screens" in "Advances in Cryogenic Engineering." Edited by: Kettel, P. Plenum Press, New York (In Press).

Lewis, M., Kuriyama, T., Xiao, J.H., and Radebaugh, R. "Effects of regenerator geometry on pulse tube refrigerator performance" in "Advances in Cryogenic Engineering." Edited by: Kittel, P. Plenum Press, New York (In Press).

Modeling, Monitoring and Fault Diagnosis of Spacecraft Air Contaminants

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Funding:

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Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$119,231

Task Description:

This project on fault diagnosis of spacecraft air contaminants has five main tasks: modeling, sensor location, monitoring, fault diagnosis, and health risk evaluation. The results of this research are critical for the on-line assessment of air quality. We will be developing a system that can make early detection of the fact that a contamination accident has occurred and give estimates of the spatial location of the contamination source and its characteristics.

We have continued our development of an intelligent system for air quality monitoring and early detection and diagnosis of air contaminants. Optimal identification of contaminants is based upon the use of an Implicit Kalman Filter, developed as part of this research, which uses both experimental measurements and a theoretical model to obtain optimal estimates. This past year we have developed a three-dimensional steady-state model of cabin air flow and a three-dimensional unsteady-state model of contaminant transport. The optimal contaminant estimates are used as the basis for the detection of a contamination event. Finally, an algorithm has been developed to allow for the location of a contaminant release and the determination of its capacity.

Safe air is a vital environmental requirement for crew members during space missions. The main objective of this research project is to develop an intelligent monitoring system capable of detecting and diagnosing contaminant emissions. To do this, we are developing an accurate model of contaminant release and transport, a detection system that uses both process information and sensor information, an optimal selection procedure, and a technique for determining the location and capacity of release events.

This research on modeling, monitoring, and fault diagnosis of spacecraft air contaminants can be applied to other air contaminant situations such as large buildings, submarines, and surface ships.

FY97 Publications, Presentations, and Other Accomplishments:

Ramirez, W.F. "Detection and diagnosis of aircraft contaminants." NASA Workshop on Environmental Space Safety.

Ramirez, W.F. "Square root implicit Kalman filtering." IFAC 13th World Congress.

Ramirez, W.F. and Skliar, M. "Detection and diagnosis of air contaminants in spacecraft." Paper 97ES-77, ICES (1997).

Skliar, M. and Ramirez, W.F. "Air quality monitoring and detection of air contaminants in an enclosed environment." J. Spacecraft & Rockets, 34, 522-532 (1997).

Skliar, M. and Ramirez, W.F. "Implicit Kalman filtering." Int. J. Control, 66, 393-412 (1997).

Capillary Electrophoretic Methods for Monitoring Spacecraft Water Quality

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 114,000

Responsible NASA Center: Johnson Space Center

Task Description:

This task is a three year program designed to apply capillary electrophoresis (CE) to the problem of detecting chemical contaminants in reclaimed drinking water. This effort will test the feasibility of CE as an inflight water quality monitor for spacecraft by developing specific analytical methods and microgravity-compatible procedures to meet the requirements of NASA's potable and hygiene water requirements for the International Space Station (ISS). CE instrumentation and procedures are inherently microgravity compatible, mechanically simple, and require minimal quantities of sample and electrolyte. The first phase of this task included extensive anion and cation methods development. It has progressed to the point where the methods are now in place for the analysis of 80% of the target compounds. Although the investigators will continue methods development throughout the course of this work, phase 2 will be the main focus of the development for this year. Phase 2 involves the development of the actual hardware necessary for microgravity-based analysis. This work includes the design, construction, ground based testing, and KC-135 based testing of the CE and associated hardware.

In principle, the separation mechanism in CE is independent of gravity and the methods and procedures developed for flight use can also be adapted for ground use and vice-versa. There are three general sectors that can benefit from the technology being developed by the investigators: the environmental laboratory, the clinical laboratory, and the ultrapure chemical industry. The environmental analytical laboratory can and does already benefit from the products of this research program. CE methods for EPA and NASA-regulated water contaminants have been used to analyze water samples from a variety of ground and space applications. The Water and Food Analytical Laboratory (WAFAL) at NASA/JSC uses these methods routinely for the analysis of drinking water, waste water, and reclaimed water samples. Routinely monitored contaminants fall into three classes: 1) small organic acids and amines, 2) common inorganic anions and cations, and 3) transition metals. CE is both a routine instrument and a niche tool for special or difficult analyses and is capable of low to mid ppb ($\mu\text{g/L}$) detection limits for the classes of compounds mentioned above. The methods development being performed by the investigators has allowed the WAFAL to add 9 new compounds to its list of routinely monitored contaminants. CE can potentially be adapted as a rapid clinical laboratory diagnostic tool due to its rapid analysis times and minimal sample requirements. Many major and minor constituents of blood plasma/serum and urine are amenable to CE analysis. CE provides many ways to overcome matrix effects such as protein adsorption that

can interfere with a given determination. CE is the ideal rapid screening tool for QA QC in ultrapure chemical or biochemical production industries. For example, the semiconductor industry relies heavily on ultrapure solvents including water for cleaning operations. Using CE's electrokinetic injection mode, it is possible to rapidly detect sub-ppb contaminants in water and other solvents. In conclusion, CE fills the voids in the analytical schema left by the established tools. Very little sample is consumed and the results are obtained in minutes. Work to improve virtually any aspect of this technology, especially the miniaturization and bubble exclusion work currently being performed by the investigators, can benefit both NASA and commercial users.

FY97 Publications, Presentations, and Other Accomplishments:

Orta, D., Mudgett, P.D., Ding, L., Drybread, M., Schultz, J.R., and Sauer, R.L. "Analysis of water from the space shuttle and Mir space station by ion chromatography and capillary electrophoresis." *J. Chromatography* (In Press).

Orta, D.R., Levy, R.J., Mudgett, P.D., Homan, M.E., Schultz, J.R., and Sauer, R.L. "Capillary electrophoresis for spacecraft drinking water analysis: Methods and breadboard development." *Proceedings of the 27th International Conference on Environmental Systems*, SAE Paper 972464 (June, 1997).

Micro-Mass Spectrometer for Contaminant Gas Monitoring

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Funding:

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Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$175,000

Task Description:

The human missions to the space station and to the planets will be of longer duration than missions undertaken so far. Activities in the habitat and outgassing of materials will release harmful chemicals in the enclosed atmosphere. The small concentrations of toxic chemicals could build up to hazardous levels in long term habitations. It is critical that the habitats of the astronauts be monitored for such chemicals. Development of a sensor to detect small quantities of these toxic/hazardous chemicals is, therefore, needed.

The objective of this research is the development of a small, low power, low maintenance sensor for monitoring the air quality in human exploration and planetary habitats. The sensor will be based on the technologies of a micro-mass spectrometer (MMS) and an array detector. The MMS uses a miniature double sector (magnetic and electrostatic sectors) analyzer (Mattauch-Herzog geometry) designed and developed at JPL. The array detector technology includes metal anodes deposited on silicon wafer along with its associated electronics, and an array detector based on active pixel sensor (APS) elements. The combination of the two technologies will produce an instrument with high sensitivity (ppb concentration level) and specificity. The proposed MMS will be compact, reliable, compatible with microgravity, and have a long operational life. The mass spectrometer will weigh 0.5-0.7 kg, and consume 1-2 W of power.

The development of the proposed instrument will constitute a major technology advancement for an *in situ* chemical analyzer. It will find other important applications in the measurement of planetary atmosphere and planetary surfaces for future NASA missions (e.g., Discovery Missions, Mars Program). Such a miniaturized mass spectrometer is also much needed for environmental and industrial process measurements.

The miniature mass analyzer has been fabricated and its performance is being characterized. We have successfully overcome the large size and weight problems historically associated with a magnetic sector by using emerging magnetic materials and novel design features for the analyzer. High energy product magnetic materials were used in the design of the magnetic sector. The electrostatic sector was fabricated from a single piece. The ion source, ESA, and the magnetic sector are all mounted on a single plate. This arrangement facilitates their alignment and makes the analyzer compact and rugged. The analyzer has a dimension of 10 cm x 5 cm x 5 cm, and weighs about 230 g.

The performance of the mass analyzer is being characterized by mass spectral measurements on this instrument. A resolution of > 300 has been obtained. In these preliminary measurements, a single element detector was used. The details of the mass spectral measurements can be found in the publication cited below. For the simultaneous measurements of all mass ions, array detectors are being developed. Work is being performed on two different types of array detectors. These include an array detector fabricated by metal anode deposition on a silicon wafer along with its associated electronics. Different elements of the detector are fabricated on the same chip using CMOS technology. The detector can be directly interfaced with a computer. The other type of array detector being developed in our laboratory is based on the technology of the active pixel sensor. Here the light collecting diode is being replaced by metal strips to collect ion images.

We have also been working on the feasibility of using filaments of different metals and oxide materials to minimize the power needs for the thermionic emission of electrons. Initial results in our laboratory show that an oxide coated metal filament can produce 300 A of electron emission with an input power of $0.6\text{-}0.7 \text{ W}$. A conventional ion source needs $3\text{-}4 \text{ W}$ of power for the same emission.

The miniature mass spectrometer when combined with a small GC will make a truly portable GC-MS system for on-site, real time measurement of environmental pollutants. At the present time such measurements are generally performed by collecting a sample and sending it to an analytical laboratory. The analysis of contaminated samples away from the site delays analytical results. A large number of such samples are often found uncontaminated and, hence, the method has proven to be costly. The delay entails inefficient use of manpower and equipment used for characterization and remediation of sites. On-site analysis will overcome this problem. Analysis of indoor atmosphere can be performed by the use of this portable analyzer. Indoor pollution has been recognized to be a major health concern.

The mass spectrometer can be applied to various other measurements which are important for public health (e.g., chemicals at industrial sites, process control, toxic waste sites, and workplace and chemical spills). So far, the unwieldy nature of a mass spectrometer (due to its large size, mass, and power requirements) has limited the use of this powerful analytical instrument to laboratories.

FY97 Publications, Presentations, and Other Accomplishments:

Sinha, M.P. "Miniature mass spectrometer (MMS) for contaminant gas monitoring." Proceeding of the 27th International Conference on Environmental Systems, SAE International, Lake Tahoe, NV (July 14-17, 1997).

Liquid Phase Piezoelectric Immunosensors

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

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Task Description:

Piezoelectric crystal sensors offer excellent sensitivity and design simplicity that make them suited for space technology. The early applications of the respective sensors were limited to measurements in the gas phase. However, subsequent technological advances including immobilization protocols and improvements in oscillator circuit designs that facilitate oscillation in the liquid phase have led to a rapidly rising interest in piezoelectric immunosensors. The overall objective of the project is to develop a piezoelectric immunosensor for *E. coli*, a representative bacteria, using antibodies as coatings. The research plan includes the evaluation of several antibody immobilization techniques, design and evaluation of a suitable oscillator circuit, and the design of a sensor array in conjunction of a flow injection system.

After extensive experimentation with oscillators circuits capable of under-liquid oscillations, two integrated chip based circuits (using SN7404 and MC10216P) were developed. These circuits demonstrated outstanding 'under-liquid' oscillations capabilities. These circuits were found not only to oscillate a piezoelectric crystal with liquid on one side, but could oscillate crystals completely immersed in distilled water. Multiple sets of these circuits were built and were integrated to serve as multi-oscillator device in a Computer Automated Biosensor Array (CABA). The CABA consists of a) multi-oscillator device, b) a multi-channel frequency counter, c) computer hardware and software to monitor the frequency, and d) pattern recognition software to classify or fingerprint the unknown agent based on the previously inputted raw data.

Despite satisfactory response of the antibody-coated crystals to isolated antigens (*E. coli*), response to samples with multiple antigens (different bacterial strains) needs to be evaluated. For this process, various combinations and types of antibodies and antigens that result in a detectably different frequency shift will be pursued. Upon successful completion of this task, the results will be used to serve as a training set for Multivariate Data Analysis. A pattern recognition software can then be used to classify or fingerprint an unknown antigen based on its response on piezoelectric crystals coated with different antibodies (or other proteins). A Scanning Electron Microscope is being employed to substantiate and/or quantify the antibody-antigen binding process.

The proposed technology can be adapted to monitor various pathogens that may cause diseases and/or affect the quality of life. Benefits may include possible applications for space, clinical, environmental, and food analysis. The successful technology will be useful to several state and federal regulatory agencies. With respect to the CABA, as multiple sensors with different protein and anti-body coatings can be used, the effect of cross reactivity (e.g., attachment of bacteria other than *E. coli*) can be ascertained using the advanced multi-variate data

analysis tools. If proven successful, this advancement in fingerprinting technology would not only be an advancement in bacterial detection underwater, but can also be extrapolated to various biosensor techniques that are presently hampered by cross reactivity.

FY97 Publications, Presentations, and Other Accomplishments:

Theegala, C.S., Berhane, B.T., and Suleiman, A.A. "A piezoelectric crystal immunosensor for *E.Coli*." SAE Technical Paper Series, ISSN0148-7191. Presented at the 27th International Conference on Environmental Systems, Lake Tahoe, NV (July 14 - 17, 1997).

Air Quality Monitoring Sensor Using Open Path Fourier Transform Infrared (FTIR)

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Funding:

UPN/Project Identification: 199-04-17-23

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Task Description:

Some key characteristics and capabilities of open-path Fourier transform infrared (OP-FTIR) spectrometry were evaluated for potential application to the measurement of trace contaminants in spacecraft air systems. The results of this evaluation suggest that OP-FTIR has the potential to provide simultaneous, near real-time quantification and confirmation of identity for most contaminants on the current spacecraft maximum allowable concentration (SMAC) list. In addition, the open-path measurement mode may afford a unique means to monitor the volumetric composition and distribution of air contaminants throughout spacecraft air systems. Compounds in the spacecraft are simultaneously monitored in real-time at high ppb to percent levels using OP-FTIR.

FTIR spectrometry utilizes an interferometric-based measurement in which data for all wavelengths are collected simultaneously. FTIR observes all compounds with characteristic vibrations between 4000 and 400 cm^{-1} (2.5 to 25 μm). Interferometers have been designed for operation in microgravity conditions. Interferograms are converted to the more familiar spectrum via Fourier transform analysis. Collection of data is independent of calibration and analysis and provides a continuously renewed database for near real-time analyses as well as for archival purposes.

The informational needs dictate the approach and methods for data reduction and analysis. Data reduction can be done in stages of increasing complexity and sensitivity to facilitate use of rugged and adaptable algorithms for extracting concentration profiles for each target compound.

Most organic and inorganic compounds have unique vapor-phase infrared signatures that can be observed for concentrations from the low ppm to high ppb levels. To evaluate applicability for the SMAC list compounds, a commercially available infrared spectral library was used which contained 111 of the 198 vapor-phase compounds. Two complementary pathlengths were identified to assure that most compounds would be observed

at the 1 hour and 7 day SMAC exposure limits. The combination of pathlengths offering the most thorough analysis of the SMAC compounds is a 15-20 cm path and a 25-40 meter path. Multiple paths can be covered using a single instrument and reflective optics or multiple instruments.

OP-FTIR collects data along the entire measurement path rather than at a single point, and thus provides a description of the volume monitored. The open-path has no walls and permits the flow of the ambient atmosphere through the optical path. Measurement along an array of paths and subsequent analysis of these paths permits tomographic reconstruction of the spatial distribution and dynamic flow characterization of air constituents and contaminants.

The study described was performed during FY97 and the original goals of the study were met and surpassed by the work accomplished. OP-FTIR monitoring of the compounds of interest at the concentrations of interest was shown to be appropriate as a sensor for consideration by NASA. The technique requires no active sampling thus avoiding the associated consequential losses and data skewing of measured concentrations of trace components.

The open literature was examined for studies in which OP-FTIR was used to monitor compounds in environments comparable to spacecraft. The pathlengths required for monitoring at the SMAC list exposure concentrations were determined for both 1 hour and 7 day exposure levels. The pathlengths required for monitoring the suite of compounds are, in general, one at 10 to 15 centimeters and the other path at 25 to 40 meters. The pathlength range appropriate for each compound at the 1 hour and 7 day SMAC levels are tabulated in the final report of this study. These pathlengths can be achieved using mirrors to direct the beam through the spacecraft and are quite reasonable. The types of data reduction algorithms and appropriate hardware for spacecraft monitoring were investigated and described.

It was shown by the study that OP-FTIR may be used to provide the baseline trace contaminant measurements for most of the SMAC list species. The ability to collect and analyze data at several levels of detail and several sensitivities simultaneously combines with the reliable confirmatory nature of the technique and represents a unique capability worthy of further investigation.

Particular emphasis in future studies should include the exploitation of tomographic modeling approaches to revealing and understanding the formation and flow of trace contaminants in life support of planetary bases and long-term space exploration vehicles. A comparison study and trade-off analysis should be done for the various analytical techniques considered for each monitoring scenario. The specific hardware requirements for the system should be outlined in detail and a prototype OP-FTIR system set up to perform tests in an appropriate chamber. The initial tests will provide data sets that may be used for data reduction studies. The compounds on the SMAC list should be ranked by order of mission-specific importance and the relative importance of long or short term analysis for each compound should be described. This information should be used in the design and implementation of the data collection and data analysis methodologies.

The appropriate spectral and time resolution should be determined for this monitoring technique. The time resolution may not have been thoroughly evaluated since other monitoring techniques have not offered such a broad range of monitoring time scales. The optimum spectral resolution will be determined by the data reduction algorithm used and the hardware requirements. Laboratory assessments may be done in a calibration cell, but tests should be performed in an appropriate chamber. The simulated environment should best mimic the environment anticipated during travel.

The progress to date completes the requirements for Arthur D. Little's contract with NASA. Future work would be completed with additional funding. A proposal for additional funding has been submitted to NASA.

This study seeks to develop a monitoring technique that is appropriate for the compounds of interest at the concentrations required by NASA as outlined by the SMAC list. The monitoring technique will be applicable to other scenarios on Earth. These monitoring applications might include: indoor air monitoring for industrial hygiene applications, assessment of air purification systems, monitoring of trace contaminants in gaseous environments, and as input to toxicological determinations of allowable concentrations. In addition, the

techniques employed are generally applicable to outdoor monitoring along the ground for environmental assessments such as fugitive emissions, stack emissions, and fence-line monitoring.

The safety and ruggedness requirements for monitoring on Earth are not likely to be as stringent as for space missions. The spacecraft is a contained vessel without interaction with other environments as is the case with many monitoring applications on Earth. The closed-system to be monitored by NASA offers an ideal situation suited for this monitoring system. The improvements in size and ruggedness to the available hardware systems that will accompany this effort will be used by many commercial and government agencies with monitoring needs that can be solved by FTIR.

The system developed will be used to determine the constituents of air in real-time. The flows of trace analytes and sources of them may be determined using tomographic modeling. Information about the types of impurities encountered in a building may be used to develop better or more appropriate air purification systems. Simultaneous detection of air constituents may also be used to correlate events and determine the sources of the trace level impurities. This type of monitoring is important in any building where people are working for long periods of time because air purity is essential for the long-term health of workers. Since the quality of breathing air directly impacts the health of workers, it impacts the bottom line of the company housed in the building.

FY97 Publications, Presentations, and Other Accomplishments:

Tucker, M.D., Rowe, R.C., Miseso, E.V., and Valentine, J.R. "Air quality monitoring by open path fourier transform infrared (FTIR) spectrometry." SAE Technical Paper Series, Paper 972391 in the proceedings of the 27th International Conference on Environmental Systems, Lake Tahoe, NV, July 14 - 17, 1997 (In Press).

Multigas Sensor for Advanced Life Support

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Funding:

UPN/Project Identification: 199-04-17-20

Solicitation: 93-OLMSA-07

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Task Description:

The work focused on the development of an electrochemical multigas sensor for air revitalization during future long duration space missions. Advanced sensors are required for such missions to monitor the levels of oxygen (O₂), carbon dioxide (CO₂), humidity (H₂O), and volatile organic compounds (VOCs) in advanced regenerative Life Support Systems. These sensors must be lightweight, compact, rugged, reliable, and consume little power to satisfy mission requirements. A sensor with these attributes would also be extremely valuable in Portable Life Support Systems to monitor system performance. The unique and innovative non-aqueous electrochemical sensor technology studied under this contract has the potential to satisfy these needs.

The amperometric electrochemical sensor technology utilizes a non-aqueous, stabilized electrolyte which will allow simultaneous detection and monitoring of O₂, CO₂, H₂O, and volatile organic compounds in an air stream. The use of the non-aqueous electrolyte allows the sensor to be operate over a wide range voltage potentials from -2.5 to +3V. This range encompasses the oxidation/reduction potentials of the desired substances to be detected.

The sensor uses a standard three electrode cell configuration. The third electrode is a reference electrode and is generally added when the conductivity of the electrolyte is relatively low or when greater precision is required.

Project Phases and Tasks

Activities for this project were divided into two phases: a Basic Contract and an Optional Phase. The Basic Contract lasted from November 3, 1995 to November 2, 1996. The specific tasks for the Basic Contract are listed below.

- Design and fabricate laboratory cell
- Test the laboratory cell's ability to detect oxygen, carbon dioxide and concentration of water (water vapor) individually and in mixtures
- Test the laboratory cell's ability to detect a variety of VOCs
- Design and fabricate a breadboard electrochemical cell

- Develop the control algorithm required to operate and test the breadboard cell
- Perform preliminary evaluation of the breadboard cell functionality

During the final quarter of the Basic Contract, NASA funded the Optional Phase which lasted from November 3, 1996 to May 10, 1997. The tasks for the Optional Phase focused on breadboard cell feasibility demonstration and are listed below.

- Assemble ceramic breadboard cell from machinable ceramic
- Perform single species testing with a breadboard cell
- Perform multigas testing with a breadboard cell
- Perform VOC testing with a breadboard cell
- Determine improvements to breadboard cell

During FY 97 the Optional Phase of the contract was performed. During the first quarterly period of the Optional Phase, work focused on assembling the ceramic breadboard cell and testing both the LCP and ceramic breadboard cells. Highlights of this work are listed below:

- Prepared experimental set up and breadboard cell for tests
- Assembled ceramic breadboard
- Conducted humidity detection tests with LCP breadboard cell
- Conducted tests with ceramic breadboard cell on the simultaneous detection of oxygen, carbon dioxide, and water vapor

Second quarter of the Optional Phase represents the final quarter of the research project. During this quarter, work concentrated on proof of concept tests with both breadboard cells. The objective was to obtain experimental results to support the use of this cell for multigas sensing in space applications, specifically in life support applications. Several sets of tests were conducted to prove the cells operational capability to detect the N₂, O₂, and CO₂ mixtures with the oxygen and carbon dioxide concentrations between 0 and 21%, and between 0 and 1% respectively. Tests were also conducted to prove the cells operational capability to detect VOCs in the ppm level. Highlights of this work are listed below:

- Refined potentiostat algorithm
- Tested the LCP breadboard cell with O₂, CO₂, and N₂ mixtures
- Tested the ceramic breadboard cell with VOCs

The whole project was completed and the final report with detailed description of accomplishments, together with conclusion and directions for the following work, was submitted to NASA HQ in May of 1997.

Summary and Conclusions

This research provided the first step in developing a nonaqueous, electrochemical, multigas sensor for use in advanced life support systems. Initial testing of a laboratory cell demonstrated that a nonaqueous electrolyte based, electrochemical multigas sensor is capable of detecting oxygen, carbon dioxide, and humidity levels fairly distinctly. This testing provided the confidence in the technology needed to develop a breadboard cell while testing of the breadboard cell proved the concept could simultaneously detect at least two gasses, oxygen and carbon dioxide, and possibly more.

The testing also indicated that the diffusion rate into the cell, specifically of water vapor, is difficult to control. In many of the tests the diffusion rates of the laboratory and breadboard cells were low. As a result, the time for the electrochemical cell to reach equilibrium with the environment was substantial. The equilibrium time, i.e. the response time, for detecting oxygen and carbon dioxide with the LCP breadboard cell was measured to between 10 and 15 minutes. The slow response could prevent the cell from reaching equilibrium in a reasonable amount of time and thus would provide inaccurate and inconsistent readings.

However, the LCP breadboard cell could readily detect both oxygen and carbon dioxide mixed with nitrogen when allowed to reach equilibrium. Changes in gas concentration produced measurable, repeatable changes in the cell's current response. The peak currents associated with the reduction of these gasses were observed to vary linearly with the individual gas concentrations. This result is extremely important to developing a usable multigas sensor.

Testing with the breadboard cell also indicated that cyclic voltammetry, as opposed to scanning voltammetry, step voltammetry, or chronoamperometry techniques, provided the most stable, repeatable current responses.

The research indicated that water vapor affects the cell's current response for oxygen and carbon dioxide. The interaction makes it more difficult to monitor the levels of the three in a mixture. Testing proved a cell could readily detect these three components in a mixture. Testing of the laboratory cell also provided an indication how the water vapor interacts with the electrolyte and alters the cell response for oxygen. This interaction was not a linear relationship as would be expected and so more testing is required to fully understand the interaction.

Testing proved the electrochemical cells could individually detect a wide variety of VOCs. The electrolyte solution used to detect the VOCs had to be of the highest purity to provide acceptable results. The testing also indicated that the redox potentials for many of the VOCs of interests are very close to one another. A method must therefore be developed to distinguish between the various VOCs. It may be possible to perform cyclic scans using different reference electrodes. Testing indicated that some of the VOC's redox potentials differed when a silver reference electrode was used instead of a platinum reference electrode. These shifts may be used to develop an algorithm to distinguish the VOCs. More work is required in this area. The VOC testing with the ceramic breadboard also proved the current response is repeatable using different electrolyte solutions.

One additional conclusion not previously mentioned is that LCP is a better material choice for a cell body than a machinable ceramic for several reasons. The primary reason is that the ceramic is slightly porous. As a result, moisture can build up inside the cell prior to assembly and during operation. This complicates cell assembly and can disturb the sensor reading during operation. The LCP is not porous and so does not have this problem. The LCP is also easier to machine and is more impact resistant.

Future Research

The following research and work are suggested to build upon this initial effort. These tasks would be the next steps to develop a useful product that could be used by NASA and other government agencies, such as the DOE, and also marketed in the commercial world.

- Work on decreasing the diffusion layer and increasing the diffusion rate to decrease the sensor's response time.
- Further characterize the impact water vapor has on cell response.
- Continue testing of VOCs with the laboratory and LCP breadboard cells to develop a cell and algorithm that distinguish the various substances.
- Perform additional testing to determine how contaminants and temperature affect cell response.
- Perform additional tests on the breadboard cell to determine how contamination and degradation of the sensor over time affects its performance.
- Perform studies on incorporation of alternative sensors into a sensor set to expand sensitivity of reading depending upon materials being sensed. This approach could incorporate parallel sensor cells (modular approach) to be tailored for different applications.
- Develop compact, portable potentiostat.

This unique electrochemical sensor technology based on nonaqueous electrolytes has the potential for long life, low cost, and sensitive and reliable performance with multigas/vapor detection and monitoring.

This sensor has applications for monitoring toxic gases and vapors particularly volatile organics in homes and at work places. The sensor has applications in environmental monitoring, in industrial process monitoring and control, and in medical diagnostics for blood gas monitoring.

FY97 Publications, Presentations, and Other Accomplishments:

Venkatesetty, H.V. "Multigas sensor for advanced life support." SAE Technical Paper Series # 972389, ICES, Lake Tahoe, NV (July 14 - 17, 1997).

New Technology for Optically-Based Multifunctioned Chemical Sensors

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Funding:

UPN/Project Identification: 199-04-17-26

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$170,800

Task Description:

This research is intended to further develop a new material that has been shown to have excellent properties for micro *in situ* chemical sensor applications. Environmental and control chemical sensors are needed by NASA for advanced systems to actively monitor and assist in the control of enclosed life support systems in current Earth orbiting and future interplanetary spacecraft. This same type of sensor will be of significant use in the terrestrial environmental monitoring of various pollutant levels, both in industry and in homes and offices. The sensors that will be developed as part of this program will be a new class of multifunctional solid-state sensors capable of discriminating, with a high degree of sensitivity and selectivity, among chemical species in complex mixtures. This will be accomplished by applying the development of new highly selective solid-state sensor elements, based upon newly developed metal/silica glass technology, to the integration into fiber-optic based sensing "optrodes". These optrodes will form the primary sensing element in a prototype multifunctional environmental sensor that will ultimately become a sensing device or instrument that is designed, and built in conjunction with, industry.

Based on our previous work, which had demonstrated unique spectral sensitivity, both absorption and luminescence, of vanadium/silica xerogels to various chemical species, the first phase of this task was intended to investigate two primary technical issues that will help develop chemical sensor optrodes described in the proposal. Over the course of approximately four months of funded activity on this task in FY 97, a significant amount of progress has been made in accomplishing the initial goals. One issue intended to be explored to establish the breadth of sensing capabilities extant in the unique metal/silica xerogels is the range of transition metals that can be incorporated in the silica; a second issue is the range of concentration of these same transition metals that can successfully be bound in the desired valence state.

The first of these issues deals with the plan to investigate the range of chemical species that can be detected spectrally via fiber optrodes. Certain transition metals will form spectrally sensitive complexes with different chemical species that are of interest for detection in the NASA life support environment. By extending the ability to bind various stable transition metal species in the silica xerogel, different spectrally sensitive complexes can therefore be monitored. During the course of the first few months that this task has been underway, the successful incorporation of the group IV metals titanium, zirconium, and hafnium into silica

xerogel has been achieved. Furthermore, addressing the second issue above, higher concentrations, i.e., up to 16.5% titanium compared to 0.5% of vanadium from previous work, have been prepared and are being readied to investigate their sensitivity to various gaseous chemical species. These transition metals constitute a homologous series that are expected to exhibit a somewhat different sensitivity and selectivity to such species as hydrogen sulfide and formaldehyde whose character is different than such other species as amines and ammonia.

In order to determine the response rate of the transition metal/silica xerogels to different chemical species prior to choosing materials for optrode sensors, pore size determination was performed on the vanadium/silica material. When the vanadium concentration is increased, it was found that the material becomes highly microporous. The larger pore size, or mesoporosity, is found to have decreased, but the pore volume has also decreased. It was found that by increasing the water content during the preparation, the mesoporosity of the 0.5% vanadium/silica xerogel could be increased. This is important in determining what factors need to be considered and controlled in preparing the xerogels for optrode material response selectivity and response rate. There are alternative methods to be considered that will aid in improving pore size control.

During the next year, the progress to date will serve as the basis to proceed into three areas. First, additional xerogel materials will be fabricated containing other transition metals. Starting materials have been identified to prepare iron (both +2 and +3 valence states), cobalt (+2), nickel (+2), and copper (+2) xerogels. The purpose of preparing these materials is to expand the detectable range of chemical species, building on the degree of sensitivity of each of these transition metals to different small molecular weight chemical species of interest to NASA. Second, in addition to the water content effect on mesoporosity of the xerogel, two additional approaches will be examined. Based upon already demonstrated effects on porosity change, the use of surfactants during the preparation of xerogels will be examined. Furthermore, the preparation of microchannels in various materials, that will serve as optical waveguides, will be initiated and used in the synthesis of xerogels. This will serve not only as a means of addressing the microporosity issue as it effects the penetrability rate of chemical species to the transition metal site in the xerogel, but also as part of the original planned preparation methodology for developing a fiber optrode and a chemical sensor. And third, extensive testing of the xerogels as sensor materials will take place. This testing can proceed now that some initial materials for examination have been prepared and the parameters that need to be investigated during preparation, and that can be altered for controlling the xerogels' response selectivity and rapidity to various chemical species, have been determined. Correlations between the meso/micro pores and the response to chemical analytes will be determined, specifically with amines and formaldehyde. In addition, the analysis of combinations of xerogel sensor materials will be facilitated by use of the microchanneled waveguides which are expected to be able to contain multichannels for simultaneously retaining many test materials.

From the work in this task, progress toward developing a multifunctional chemical sensor is being made. The outcome of such a sensor will be a device that will have many uses in Earth applications. Among the most apparent are in the chemical industry for safety as well as in process control. In the normal working environment, this type of sensor device will be applicable to analyzing, in real time, the levels of various noxious or harmful chemical species that can build up under certain off-design conditions, including carbon dioxide and carbon monoxide levels, hydrocarbons from natural gas or LPG gas line leaks, etc. The sensor anticipated to be developed from this work will be easily adaptable to various environments as well, which offers the potential for a very low-cost instrument.

Crop Production Optimization Using CO₂ Gas-exchange

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-61-17-08

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 8

Post-Doctoral Associates: 1

FY 1997 Funding: \$156,489

Task Description:

We focused on 5 areas in 1997: 1) We used measurements of CO₂ exchange in sealed chambers to quantify the short- and long-term effects of primary environmental factors on daily production efficiency, canopy quantum yield, and canopy carbon use efficiency. These measurements have provided the basis for verifying and refining our energy cascade model of wheat productivity. 2) We examined the effects of blue light fraction on growth and development of lettuce, wheat, and soybeans. 3) We quantified the effect of super-optimal CO₂ levels on growth and yield of wheat. 4) We studied the effects of high levels of ammonium (85% NH₄⁺) in hydroponic solution. 5) We continued our wheat breeding studies to develop a high yielding super dwarf wheat line for use in the confined volumes of sealed environments.

Objective 1: CO₂ exchange and productivity analysis. A major paper describing these results is now in press (see publications). We expect that the energy cascade approach used in these studies will see broad application at the NASA field centers.

Objective 2: We completed an initial set of studies with blue light fractions from 0 to 25% blue. A paper describing the results of these studies is in press. There appears to be an optimum blue light fraction at about 6% blue light, but the optimum appears to depend on total PPF intensity.

Objective 3: Super-optimal CO₂. We published 2 papers on the detrimental effects of super optimal CO₂ on wheat. It now appears that the effects are caused by a reduction in respiration in the wheat heads.

Objective 4: We completed an initial set of studies comparing 0, 0.25 and 0.85 ratios of ammonium/nitrate in hydroponic solution. There was no detrimental effect of high NH₄⁺ on vegetative growth, but seed yield was decreased by 15% at the highest NH₄⁺ level.

Objective 5: We are continuing our wheat breeding efforts. We now have a series of wheat lines in 5 cm increments ranging from 25 to 50 cm tall. Yield increases with increasing height, but all the lines are improvements over previously used wheat cultivars.

This research is helping crop physiologists refine models of food production on Earth. Specifically, we can make measurements in controlled environments that cannot be made in field environments. The infrared

transducers we have developed should be of direct value to agriculture in the field. The new wheat cultivar that we have developed is not directly useful in the field but it helps us understand the limitations to yield in the field.

FY97 Publications, Presentations, and Other Accomplishments:

Bishop, D. and Bugbee, B. "Photosynthetic capacity and dry mass partitioning in dwarf and semi-dwarf wheat." *J. Plant Physiol.* (In Press).

Bugbee, B., Droter, M., Monje, O., and Tanner, B. "Evaluation and modification of commercial infra-red transducers for leaf temperature measurement." *Adv. Space Res.* (In Press).

Bugbee, B., Koerner, G., Albrechtsen, R., Dewey, W., and Clawson, S. "Registration of USU-Apogee wheat." *Crop Sci.*, 37, 626 (1997).

Dougher, T. and Bugbee, B. "Effect of lamp type and temperature on development, carbon partitioning, and yield of soybean." *Adv. Space Res.* (In Press).

Grotenhuis, T. and Bugbee, B. "Super-optimal CO₂ reduces seed yield but not vegetative growth in wheat." *Crop Sci.*, 37, 1215-1222 (1997).

Grotenhuis, T., Reuveni, J., and Bugbee, B. "Super-optimal CO₂ reduces yield in growth chamber and greenhouse environments." *Adv. Space Res.* (In Press).

Jurgonski, L., Smart, D., Bugbee, B., and Nielsen, S. "Controlled environments alter nutrient content of soybeans." *Adv. Space Res.* (In Press).

Reuveni, J. and Bugbee, B. "Very high CO₂ reduces photosynthesis, dark respiration, and yield in wheat." *Ann. Bot.*, 80, 539-546 (1997).

Smart, D., Ritchie, K., Stark, J., and Bugbee, B. "Evidence that elevated CO₂ levels and indirectly increase rhizosphere denitrifier activity." *Appl. Env. Microbiol.*, 63, 4621-4624 (1997).

Tubiello, F., Volk, T., and Bugbee, B. "Diffuse light and wheat radiation-use efficiency in a controlled environment." *Life Supp. Biosphere Sci.*, 4, 77-85 (1997).

Volk, T., Bugbee, B., and Tubiello, F. "Phasic temperature control appraised with the CERES-wheat model." *Life Supp. Biosphere Sci.*, 4, 49-54 (1997).

Space Suit Survivability

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Funding:

UPN/Project Identification: 199-71-17-01	Solicitation: 96-OLMSA-01
Initial Funding Date: 1997	Expiration: 1998
Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: \$144,761	

Task Description:

The materials developed for the Extravehicular Activity (EVA) spacesuit have provided an effective solution for protecting the spacesuit's pressure envelope from the hazards encountered in space throughout the history of the manned space program. Enhancements to the spacesuit's materials have been made each time the role of the spacesuit changed over time, such as from Gemini to Apollo, when the risk associated with the working environment increased. Recent missions suggest the "survivability" of the current Space Suit Assembly (SSA) should be enhanced to combat the increasing threat levels encountered during EVA. Increasing threat levels have been noted in the form of a greatly expanding flux of micrometeoroids and debris (MMD) that could potentially impact and puncture the SSA, and a greater manipulation of hardware with potential sharp edges that could puncture the SSA. The potential for damage from both threat types will increase dramatically with the construction and habitation of the International Space Station (ISS) as well as with the increasing role of the astronaut in satellite repair, thus indicating the need for increased survivability of the spacesuit.

Computer models of the probability of survival of the spacesuit that account for the rapidly increasing debris threat indicate that an increase in protection of the spacesuit over its current level will be required near the year 2000 (Hodgson, 1993). Several recent Shuttle missions have also witnessed damage to the spacesuit from cut and puncture threats that were of a serious nature regarding the safety of the SSA. The potential for damage from these threats is in direct correlation to the increase in quantity and complexity of EVA performed. These potential occurrences must be addressed immediately to maintain NASA's stated safety goals as we move into the Space Station era.

The primary emphasis of this research effort will be to develop spacesuit materials or systems that include provisions for self-sealing or pressure containment in the event of a penetration of the fabric pressure envelope. Secondary emphasis will be placed on identifying and utilizing materials that will provide greater defensive measures against MMD impacts as well as improve the puncture and tear resistance of the spacesuit.

Technologies developed in the space suit survivability enhancement task will find Earthbound application in the protection of humans from sharp object threats in hostile environments. This may be in the form of protective

clothing or gloves worn in the presence of objects that can easily damage skin such as knives or abrasive materials.

Another aspect of the effort is to produce self-sealing pressure barriers. This may find application in any number of inflatable/pressurizable devices ranging from aircraft fuel bladders to airships.

This program was awarded September 17, 1997, and has only just been initiated.

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Funding:

UPN/Project Identification: 199-61-17-28

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$132,834

Task Description:

Growing photoautotrophic plants in an enclosed controlled environment for the purpose of developing biologically-based Advance Life Support currently has two major challenges: first is the extensive use of highly energy-intensive artificial lighting sources, and second is the substantial energy wastes incurred through heat dissipations by these lamps, dictating an unnecessarily large, and costly, physical volume for the plant-growing structures. The long-range goal of this proposed research is to meet the criteria of energy efficiency and optimal miniaturization of plant growing structures for bioregenerative Advance Life Support by employing a Solar Irradiance Collection, Transmission and Distribution System (SICTDS) that will deliver available and heat-free solar irradiance to growing plants in a bioregenerative life support system. Our present aim is to determine the feasibility of using an SICTDS, consisting of a Himawari solar collector, fiber-optic-cable light transmitters, and three light-distribution schemes, in growing crops in remote chambers located in an enclosed Subterranean Plant Growth Facility (SPGF) in Tucson, Arizona where the average total solar radiation falls just a little below that found on the lunar surface. We will determine the efficiencies of our SICTDS as well as the ability of SICTDS-delivered light to sustain crop growth and their effects on crop physiology. In future projects, we will incorporate the more efficient parabolic-dish solar collectors into our SICTDS.

The following tasks were accomplished during FY 1997:

1. Preparation of the Subterranean Plant Growth Facility (SPGF): The Subterranean Plant Growth Facility located in an open space next to the Tucson International Airport, and within the compound of The University of Arizona's Environmental Research Center, was prepared for conducting tests on our project's SICTDS which is designed for hydroponically growing photoautotrophic crops in a remote and enclosed location. Two identical growth chambers, each with an interior volume of 1 m³, were housed within the SPGF, one for testing the SICTDS treatment and the other for running the control with high-pressure sodium (HPS) artificial lighting. The two chambers were provided with identical hydroponic growing tray systems, a shared nutrient delivery system, and a shared air ventilation system. They were provided with common liquid nutrient reservoir, pump, liquid filter, nutrient and pH adjustment systems, liquid nutrient flow rate in the growth trays,

temperature control (air cooling heat exchanger), air filter, and air flow rate. The photosynthetic photon flux (PPF) of the control is designed such that the total photons in the control is the same as the total photons in the SICTDS treatment for equivalent days at equal photoperiods. The SICTDS treatment is run two days ahead of the control to adjust both the photoperiod and the average PPF in the control accordingly. The monitoring and regulation of environmental parameters such as PPF, air temperature, relative humidity, nutrient conductivity, and nutrient pH, among others, are performed automatically using a computer system.

2. Testing of the SICTDS: The SICTDS being used in the current project consists of a fresnel-lens-based Himawari [*Japanese*: sunflower] solar collector, seven cables or bundles of fused silica optical fibers, and three different distribution schemes. The Himawari solar collector was set on top of the SPGF, and its transmission cables penetrate through the roof of the SPGF into the growth chambers wherein crops are grown hydroponically. The transmitted light is released from the transmission cables through the distribution devices and onto the plants. The distribution scheme currently being tested consists of the tips of the optical fibers emanating from the fiberoptic cables arranged in a rectangular matrix over the growing area. Measurements are currently being performed for PPF distribution, spectral distribution, transmission efficiency, SICTDS efficiency, and crop productivity in terms of both energy and physical volume utilization. The same tests will be performed using fiberoptic pads and light-emitting fibers as distribution devices and using high-efficiency plastic optical fibers as transmission systems.

The Earth benefits of this project lie in the design and development of an optimal plant growing system in terms of efficient energy consumption -- owing to the utilization of available, or free, solar irradiance -- and in terms of optimally minimized physical volume for growing plants -- owing to the use of low-heat, fiberoptic-delivered light, permitting close contact between light source and plant canopy.

AI Software Development for Advanced Life Support

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No Co-Is Assigned to this Task

Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$96,000

Task Description:

The overall objective of this project is to identify complex data processing requirements for advanced life support (ALS) and to develop advanced automation/artificial intelligence (AI) approaches where appropriate. ALS involves processing a lot of sensory data to generate control signals for a variety of processes with a wide range of characteristics. Data redundancy offers the opportunity to improve system robustness. The main justification for AI is to reduce crew time requirements for data reduction and system monitoring, management, and maintenance. By ensuring optimum system management, such as varying the environment to suit the actual stage of growth as the crop matures, productivity would be increased. However, AI, particularly expert systems, also offers the option of improving mission autonomy with reduced ground support costs as a consequence. All of these options are possible with conventional data automation, but would be more difficult and costly to develop, use, and maintain.

Work was completed on this project though the progress was slower than planned due to reduced funding; all planned tasks were not completed. Work focused on three areas: modifying OCAM to reflect BIO-Plex configurations and operational planning, further modifying the model for use with the KSC ALS Breadboard Facility (ABF), and continuing to provide limited support to replacing UNDACE with a more intelligent system. Increased detail was modeled, enhancing the basic OCAM capabilities.

BIO-Plex Modeling

Several BIO-Plex models were developed, including 1 and 2 biomass production chamber versions. A 5 chamber version of OCAM was developed reflecting the 2-BPC BIO-Plex configuration as currently defined. This model accommodated current crop information, as well as crew changes. Air transfer between modules was controlled, but this is probably not needed as long as adequate circulation is provided, and that will be simpler to implement. Key issues to be decided include the mass flowpath desired in BIO-Plex. Significant reductions in system mass can be achieved by maximizing use of plants for air revitalization and water regeneration rather than adding a bioregeneration system to a complete PC system. Even with only partial food closure, complete closure of air and water loops can be achieved.

Not considering start-up and harvest interval, complete water regeneration can be achieved with about 25% food closure, based on measurements in the ABF. This number can be reduced or increased by perhaps a factor of two according to the environmental conditions (particularly humidity and wind speed). Complete air regeneration can

be achieved if two conditions are met: if the productivity of total biomass is adequate to support crew metabolism, and if the harvest index is lower than the food closure.

The original development used the second BIO-Plex cropping scenario with 6 crop species. The model calculated that the productivity could support up to 69% food closure. An additional cropping scenario has been developed by the BIO-Plex team, including tomatoes as a specific crop as well as sweet potatoes. This scenario was calculated to be less efficient, supporting only 50% food closure.

ABF Data Modeling - Model-Based Data Evaluation

An OCAM model of the ABF was built, and data was imported from ABF files to calculate system state for a single crop. It successfully calculated crop growth based on CO₂ uptake (accounting for changes in pCO₂ and CO₂ mass flow into the chamber). A major problem was encountered in predicting the partitioning of biomass between edible and inedible biomass. For the crop used, potatoes, tuberization can be observed visually, which could potentially be automated, but partitioning evidently does not switch dramatically from one state to the other, and vegetative production was greatly underestimated with the algorithm used. Conceivably, there is some indicator that can be used to predict the degree of partitioning, but we have not found it to date. Probable indicators amenable to automation may include nutrient uptake and shoot space trace gases.

The approach of using a model to calculate system state was thus amply confirmed, and the data representation suggested that this would be an excellent way to present data to an operator, with the data arranged hierarchically. Thus, if an operator wants to see how the system is performing, a comparison of actual and predicted life support quantities (oxygen, CO₂ uptake, water production, or biomass production) does seem to be appropriate.

CMU Support

The UNDACE monitor and control (M&C) system currently used for the ALS breadboard is an orphan system, written in largely undocumented and unmaintainable C code, and dependent on an obsolete operating system to run. One of the options considered for replacing UNDACE is the KSC-developed Control and Monitoring Unit (CMU). This is a M&C system that was derived from the Partial Payload Checkout Unit that was developed and used for checking out small payloads at KSC. CMU and its derivatives have been used for other systems such as for control of the Delta Clipper, DC-X, the upgraded DC-Xa, and Delta 4 (EELV).

Support has been provided to a joint NASA/contractor working group to define CMU capabilities and strengths and to use a G2 implementation to manipulate the data. Besides being used for modeling as described above, G2 provides neural net, knowledge-based reasoning, and user interface development capabilities.

Controlled environment agriculture is becoming increasingly important as we attempt to reduce the environmental impact of agriculture and increase the quality of produce. Similar problems will be encountered with monitoring and control systems both in space and on the ground, particularly as increasing amounts of intelligence are used for control applications. This task will benefit monitoring and control systems for commercial and research environments, including both greenhouses and growth chambers. Better control will increase productivity and reduce cost.

FY97 Publications, Presentations, and Other Accomplishments:

Drysdale, A.E. "Computer modeling for advanced life support system analysis." *Life Supp. Biosphere Sci.* (In Press).

Drysdale, A.E. "OCAM-2: A second generation bioregenerative life support system model." SAE 972291 (1997).

Drysdale, A.E. and Finger, B. "Waste processing for ALS: Influences on operational strategies and design." SAE 972292 (1997).

Hunter, J.B., Lin, S., Drysdale, A.E., and Vodovotz, Y. "Prospects for single-cell oil production in a lunar life support system." SAE 972365 (1997).

Adsorbed Carbon Dioxide and Water Interactions and Maintenance of Low CO₂ Levels in Closed Environments

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Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 125,000

Responsible NASA Center: Ames Research Center

Task Description:

The current specification for the allowable carbon dioxide concentration on the International Space Station Alpha (ISSA) has caused concerns among investigators planning life science experiments on the space station. At about 0.7%, the specified maximum allowable concentration is much higher than the concentration of CO₂ found in the Earth's atmosphere (0.03%). Because of CO₂'s physiological effects, the high level of CO₂ may make meaningful comparisons between ground and flight experiments difficult or impossible.

While a new specification has not yet been established, the CO₂ removal design in the current ISSA life support system is incapable of meeting a much lower CO₂ specification, given the CO₂ generation rate of four crew members. This is primarily due to the inefficiency of the adsorption technology used to remove the CO₂. The inefficiency is caused by the processor's need to remove all water from the process air stream; over 80% of the power it draws is used for desiccation and re-humidifying the cabin. A strong, adverse interaction exists between water and CO₂ on the adsorbents used in the current design. A regenerable, high-flow CO₂ removal device would have some clear advantages over the current technology, but its development is hampered by a profound lack of basic experimental data and theoretical prediction capabilities required for efficient design. Without these, the design process is extremely expensive and risky.

This research seeks to develop a basic understanding of how CO₂ and water interact with each other when adsorbed on various hydrophilic and hydrophobic adsorbent surfaces and how the interactions affect the performance of CO₂ separation processors. The theory will be implemented in process models, which will in turn be used to make processor recommendations and designs. The research may also find application to CO₂ removal from a humid natural gas stream, CO₂ concentration and control in closed environmental chambers, and air revitalization on long-duration passenger aircraft flights.

Research in FY97 focused upon development of a hybrid membrane/sorption process for CO₂ removal. In the process, water vapor is removed from the inlet air stream using a countercurrent-flow membrane-based desiccation unit. Moisture is picked up by the exit air stream and eventually returns to the cabin, and thus much of the energy consumption caused by removing water as a separate phase (as in current CO₂ removal process

designs) can be eliminated. The air emerging from the desiccation unit has a low dew point but still contains the inlet quantity of CO₂. If necessary (i.e., if currently used zeolites are used for CO₂ scrubbing), the residual moisture in this stream can be removed before CO₂ separation. Alternatively, if a sorbent that can effectively remove CO₂ from a stream containing small amounts of water is available, then no further desiccation is necessary. We have been working on the latter design, using a solid amine sorbent provided by the Hamilton-Standard Company. This design is being implemented in Phase III closed-chamber testing at the NASA Johnson Space Center.

Carbon dioxide buildup is a potentially critical problem for maintaining breathable air in any closed environment. These environments include not only spacecraft and future planetary habitats, but modern buildings that draw in minimal fresh air for reasons of energy savings, passenger aircraft, vehicles on battlefields (such as tanks, helicopters, and personnel carriers), and underwater and high-altitude vehicles. If CO₂ removal is necessary for these applications, then it will probably also face the difficulty of efficiently removing CO₂ from humid air, the problem this research addresses. There are also industrial applications which require CO₂ removal from a humid gas stream such as CO₂ scrubbing of natural gas. The benefits these applications could see from this research are mainly smaller and more energy-efficient ways of maintaining CO₂ at more desirable levels, which in turn would have positive impacts on human health and well-being and prices of industrial products.

Cost Optimization of Food Preparation for Lunar and Planetary CELSS Stations

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Funding:

UPN/Project Identification: 199-61-17-26

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 137,178

Task Description:

Our general objective is to develop a database of food processing information and an optimization system to support informed decisions regarding food preparation in the HRTF or a lunar/Martian colony. We will develop a menu of 100+ tested recipes based on CELSS crops -- a "CELSS cuisine" of nutritious dishes that the station crew will enjoy eating during their residence in the station. The dishes will include both familiar and novel menu items. Each food will be evaluated for palatability, nutrient content, and preparation cost in terms of equivalent mass. The project will include the following components:

Development of >100 tested recipes for ingredients and foods based on CELSS crops -- the large number intended to provide future crews with a range of dietary choices, and to allow mission management to optimize the menu for closure, labor costs, or other constraints.

Evaluation of the acceptability of CELSS-compatible menu items to the astronaut population.

Estimation of nutrient content of individual dishes and overall menus using an existing software package and available nutrient data for raw materials and ingredients.

Development of information on labor and equipment costs of food ingredient manufactured from CELSS crops and on costs of food preparation from these ingredients.

Construction of a simulation model to estimate the preparation cost of each food and ingredient for any given mission, subject to reasonable assumptions and constraints.

Generation of optimized diets from the database of foods, using a linear programming package to select the lowest cost diet which meets user-supplied constraints on nutritional quality, hedonic acceptability, and specific resource limitations.

Training of future crewmembers, CELSS engineers and managers, and food service personnel in appropriate food preparation techniques to support BIO-Plex studies and future menu development efforts.

Several startup tasks were completed in FY97. The requirements for the food database were established in consultation with database developers and project participants. Database development was completed except for the security features required before the database can be opened on the Web to serve multiple contributing sites. Entries, changes, and deletions to records must be password protected to preserve intellectual ownership of individual recipes. The database includes 4 layouts per record; one each for general information and preparation

instructions, nutritional data, sensory panel testing data, and cost estimation data. General information and sensory data is entered by hand, and the nutritional and cost data are calculated by a nutritional analysis package and a spreadsheet program respectively, then imported into individual records.

A food preference questionnaire for the astronaut corps, based on previous Army studies, was abandoned when it became clear that access to astronauts as survey subjects was tightly restricted. Without authentic data on current astronaut food preferences and food preparation training, it was decided to match panelists to the astronaut corps on general characteristics: age, gender, body weight, physical activity, and education. Around 400 largely-vegetarian recipes from cookbooks, personal files, and Internet sources were reviewed to select the first hundred recipes for testing.

Testing procedures and questionnaires were developed for panel testing, and the lighting and cubicle design of the panel testing space were updated.

Research was initiated on small-scale processing technology for seitan (a meat alternative based on wheat gluten), small-scale soymilk production, and starch-based sweeteners from wheat, rice, potato, and sweetpotato prepared by the koji/amazake process and direct enzymatic hydrolysis. Appropriate small scale food processing equipment was identified and some has been received. A master's thesis was completed on production of amazake (a starch-based sweetener indigenous to Japan) and koji (a highly amylolytic solid-state culture of *Aspergillus oryzae*) from wheat, potato, and sweetpotato. A cost estimate for single-cell oil production from inedible wheat residues was presented at the 27th ICES conference.

The SPACEFOOD-L electronic mail discussion list on food systems for space flight and space colonization was established in September 1997.

This work combines the conventional application of linear programming optimization to select low-cost nutritionally balanced diets, with flexible cost estimation procedures and measures of food acceptability, and has the ability to handle ingredients and recipes numbering into the thousands. It will ultimately provide an adaptable strategy to improve human diets and menus in institutional food service settings, such as hospitals, military dining halls, correctional institutes, and school lunch programs. The planned work on sensory testing and the closed feeding study should lead to a better understanding of the acceptability of novel, low-fat, and plant-based foods to consumers of a standard Western diet. This knowledge in turn will help food manufacturers and public health organizations to promote healthier eating habits on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Hunter, J.B., Lin, S., Drysdale, A.E., and Vodovotz, Y. "Prospects for single-cell oil production in a lunar life support system." 27th ICES, SAE Paper 972365 (1997).

Yau, J.C. Study of Amazake production using different substrates. (Thesis) Cornell University, Ithaca, NY (1997).

Enhanced Oxidation Catalysts for Water Reclamation

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UPN/Project Identification: 199-61-17-27

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$76,292

Task Description:

This effort seeks to develop and test high-performance, long operating life, physically stable catalysts for use in spacecraft water reclamation systems. The primary goals are to a) reduce the quantity of expendable water filters used to purify water aboard spacecraft, b) to extend the life of the oxidation catalysts used for eliminating organic contaminants in the water reclamation systems, and c) reduce the weight/volume of the catalytic oxidation systems (e.g., VRA) used. This effort is targeted toward later space station utilization and will consist of developing flight-qualifiable catalysts and long-term ground tests of the catalysts prior to their utilization in flight. Three years of life tests and performance tests are proposed.

Noble metal-catalyzed oxidation of organic contaminants in water is highly effective for water purification. Utilization of activated carbon to support noble metal catalysts results in the highest activity catalysts currently known. Other materials show significantly less activity as supports. However, processes employing carbon-supported catalysts suffer oxidation of the support matrix, resulting in inadequate catalyst life for long-term space applications. Scientific groundwork now exists to extend the operational life of existing catalysts, particularly catalysts used in missions with limited re-supply capabilities. New surface treatments and impregnation agents have been demonstrated to be effective at inhibiting oxidation of the activated carbon support over short-term testing. Certain oxidation inhibition treatments were demonstrated to have no effect on catalytic activity of noble metal/carbon catalysts during short-term tests. It is the purpose of this effort to take advantage of these new developments by performing life and parametric tests, and comparing the performance and life of the new oxidation-resistant catalysts with the currently used activated carbon- and alumina-supported catalysts.

This project was initiated late in FY97 and is in its preliminary stages. Environmental and Life Support Technologies Inc. is currently preparing materials for testing in-house for space applications and at a major industrial firm for pharmaceutical industry applications.

This program is directed toward 1) a new technology for improving existing process cost efficiencies, and 2) a new class of catalysts for air and water treatment that will improve process economics and provide a valuable tool for industrial chemical processing, water and air treatment, pollution control, environmental remediation, and ultra-pure water production. Over 6,000 world-wide applications are estimated.

ELS has signed agreements with the two major industrial manufacturers of carbon-supported metal catalysts in the U.S. to evaluate this technology. One of these manufacturers has begun bench-scale evaluation of catalyst materials for five industrial processes in environmental treatment and pharmaceutical products each with product sales in excess of \$100M per year. ELS is currently preparing materials for these evaluations.

Enhanced Molecular Sieve CO₂ Removal Evaluation

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Funding:

UPN/Project Identification: 199-61-17-11

Solicitation: 93-OLMSA-07

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The objective of this research was to quantitatively characterize the performance of two major types of molecular sieves for two-bed regenerative carbon dioxide removal systems at the conditions compatible with future IVA and EVA missions. The first sorbent is a zeolite-based molecular sieve that has been substantially improved over those in used in Skylab. The second type is a recently developed carbon-based molecular sieve based on a carbon adsorbent. Both of the molecular sieves offer the potential of high payoff for future manned missions by reducing system complexity, weight (including consumables), and power consumption in comparison with competing concepts. The research provides the technical data enabling improved CO₂ removal systems for regenerative life support systems to be developed.

The investigations of the CO₂ materials under dynamic conditions were successfully performed:

- The different versions of the materials (zeolite and FCMS) were characterized via isotherm curves and newer variants show a capacity improvement over previous generations.
- A test rig capable of open and closed loop operation with computerized data acquisition was configured and used for test.
- Investigations of packing density, pressure drop, and flow distribution were performed.
- An analytical model of the performance of the pressure swing system in different thermal modes was developed and evaluated against test data.
- Cyclic testing of the sorbent materials in two differing bed designs under space station and spacesuit conditions showed repeatable and consistent performance at all test conditions investigated. Independent variables tested included half cycle duration, flow rate, total pressure, partial pressure of CO₂ and water, test bed aspect ratio and total volume, and operational protocol. These tests demonstrate the operation of a CO₂ removal system using only pressure swing to regenerate the material.
- The test data were used to develop system concepts for both suit and station applications for evaluation against current technologies. The sorbents tested, in a pressure-swing system, were competitive for both applications.

The primary goal of this research is to investigate a new technology for the selective removal of CO₂ from air (or oxygen) for space applications.

Selective CO₂ removal also has terrestrial applications, notably for food storage, production plant cleanup, submarine air revitalization, and medical applications. Additionally, CO₂ removal from air is related to global warming, and in the future, control of waste gas emissions of CO₂ may become necessary. This research also helps define the chemical processes and conditions required for these types of control systems.

The most important gains of this study may be to facilitate man living in a closed environment by characterizing new CO₂ selective sorbents under different conditions with different bed designs and operations.

FY97 Publications, Presentations, and Other Accomplishments:

Rose, S.K., MacKnight, A.K., and El Sherif, D. "CO₂ removal with enhanced molecular sieves." SAE-972431; International Conference on Environmental Systems, Lake Tahoe NV (July 14 - 17, 1997).

Control Systems Integration in Closed Ecological Systems Using Denitrification as a Model

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Funding:

UPN/Project Identification: 199-61-17-29
Initial Funding Date: 1997
Students Funded Under Research: 0
FY 1997 Funding: \$145,584

Solicitation: 96-OLMSA-01
Expiration: 2001
Post-Doctoral Associates: 0

Task Description:

Two fundamental issues relevant to the Advanced Life Support research program, as described in NRA 96-OLMSA-01B, include system mass balance and system control issues. The research proposed here addresses both of these issues, by coordinating and integrating technology development (e.g., autonomous control of a bioreactor) with experimental research (i.e., loss of fixed nitrogen through denitrification). This will be accomplished by testing the following hypothesis and meeting the specific objective of the proposed research. Our hypothesis is that the loss of fixed-nitrogen via denitrification in plant growth chambers used in advanced life support systems can be greatly reduced through the development of autonomous control strategies for controlling the availability of O₂ to denitrifiers. The approximately 30% loss of fixed nitrogen represents a significant loss for advanced life support systems. This is supported by data collected from the plant growth chambers at Kennedy Space Center (KSC). The availability of O₂ is known to affect denitrification and its end-products. The specific objective of the research is to use the loss of fixed-N by denitrification in closed systems as an example of how to develop autonomous control systems for a bioreactor, and to extend this example to control issues relevant for other important processes within advanced life support systems. Meeting this objective fulfills both science and technology goals, and tests the hypothesis. The hypothesis will be tested and the objective met by increasing the complexity of the software, hardware, and microbe portions of our current system to achieve a close approximation of the parameters important to controlling denitrification. Development of control systems for ALS systems will be enhanced by using control of denitrification as a model. This plan culminates in physically transferring to, and testing the denitrification control system on, a plant growth chamber at KSC. We will begin with well-characterized organisms (*Paracoccus denitrificans* and *Pseudomonas fluorescens*) grown alone, then increase the complexity to a two organism mixed population in a defined medium, incorporating the data into the feed-back and feed-forward control system software architecture. The next series of experiments will use known organisms grown in a standard plant growth medium (half-strength Hoaglands), followed by growth in medium collected from a plant growth chamber at KSC. The final series of experiments will use inocula from a plant growth chamber grown in our bioreactor on half-strength Hoaglands. At each stage, data will be used to refine the simulation models and control system features.

Denitrification, the dissimilatory reduction of NO₃⁻ to N₂O and N₂, is found in a wide variety of organisms. In closed artificial systems, especially closed plant growth chambers, significant amounts of fixed nitrogen is lost through denitrification, thereby decreasing the efficiency of the system and fouling the atmosphere with N₂O.

Denitrification is a form of anaerobic respiration. Whenever available, however, denitrifiers preferentially use O_2 as their terminal electron acceptor. As a result, rates of denitrification and growth are a function of pO_2 . Typically, in closed systems O_2 consumption is greater than the diffusion of O_2 through the medium to the cell, decreasing the pO_2 near the cell and denitrification occurs. Another parameter controlling denitrification is the availability of NO_3^- . Using *Pseudomonas fluorescens* (ATCC #17400) grown in a 2L bioreactor, we controlled and modeled the population growth and rate of denitrification as a function of pO_2 , an environmental parameter that mitigates denitrification. *P. fluorescens* does not denitrify and has a generation time ($t_{1/2}$) of 5 hr when grown aerobically; whereas, when the $pO_2=0$, the $t_{1/2} = 23$ hrs and the rate of denitrification increases. Our hypothesis is that this loss can be greatly reduced through the development of autonomous control strategies for controlling the availability of NO_3^- and O_2 to denitrifiers. The design features of the system include real-time monitoring and control of experimental parameters (e.g., temperature, pH, NO_x , CO_2 , and cell density). Control of key system parameters is achieved by incorporation of artificial intelligence software tools that permit autonomous decision-making by the system. Traditional hardware control is implemented in Labview which posts system information across a TCP/IP link to the AI software. Several AI components (e.g., Livingstone and PROPEL) have been integrated into the software hierarchy which permit quantitative and qualitative modeling of system behavior, thereby enabling fault diagnosis, prevention, and recovery, via real-time revision of control laws.

The research being performed under this task provides Earth benefits in two areas. First this research will yield information on basic biological processes related to nitrogen cycling, especially the process of denitrification. Denitrification occurs in a wide variety of natural and human-influenced (e.g., agricultural) ecosystems and contributes significantly to the loss of fixed nitrogen from many of these systems. One denitrification by-product, nitrous oxide (N_2O) acts as a greenhouse gas in the troposphere and in the stratosphere it contributes to the destruction of the Earth's ozone layer. The basic research conducted here to control denitrification in bioreactors and closed plant growth systems may find application in non-laboratory settings. Specifically, improved understanding of factors (e.g., pCO_2 , pO_2 and temperature) that affect rates of denitrification, and of the interactions (competition) between nitrogen-transforming bacteria, will be useful in devising strategies for controlling denitrification as a part of comprehensive land-use strategies. Such strategies may benefit the Earth in terms of reduced use of nitrate fertilizers, with attendant cost savings by farmers and a reduction in nitrate runoff into aquatic systems. Further, the control of N_2O production will aid in reducing global warming and rate of destruction of the ozone layer. The second Earth benefit derives from development of new control technologies based on both conventional and artificial intelligence software techniques. The integration of AI software tools with conventional control system technology is a critical step in the development of autonomous instrumentation. Such technology permits untended operation of science instruments and industrial processes. Improved fault diagnosis and recovery is important for long-term robotic space missions as well as protection of personnel and equipment in conventional industrial settings. Specifically, one may envision this technology being used on deep space probes that cannot rely upon human control, and in a variety of industrial processes where human intervention is limited by safety, or personnel availability considerations.

Membrane Based Thermal Control Development

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Funding:

UPN/Project Identification: 199-71-17-02 Solicitation: 96-OLMSA-01
Initial Funding Date: 1997 Expiration: 1998
Students Funded Under Research: 0 Post-Doctoral Associates: 0
FY 1997 Funding: \$113,010

Task Description:

This research task addresses the Extra Vehicular Activity (EVA) element of Advanced Life Support. The proposed project will determine the feasibility of a membrane-based thermal control device. A membrane device that separates liquid water from the vacuum of space could provide cooling by evaporating the liquid. The project will include testing of candidate materials for water transport and transfer characteristics. Viable materials will be further tested for mechanical durability to determine if they are suitable for an EMU application. The product of this research will be a report which will address the findings of the performance testing and conclusions drawn from the system sizing effort. The report will assess the overall feasibility of this approach.

A variety of different membrane materials, both hydrophilic and hydrophobic, were screened to determine the feasibility of using them for this thermal control application. The screening test consisted of introducing water to the surface of the membrane surface and observing how much water, if any, wept through the membrane at differential pressures up to 15 psid. The samples that passed the screening test were performance tested.

Performance testing consisted of measuring the rate of water evaporating through the membrane; water flowed past the surface of one side of the membrane with the other side exposed to vacuum. Temperature measurement of the water stream in and out of the membrane holding device indicated the rate of heat transfer provided by the membrane. This value was correlated to a measurement of the amount of water lost from the water supply.

Overall, the hydrophilic membranes had a higher heat transfer rate than the hydrophobic membranes. These membranes will be subjected to a set of endurance and contamination testing. Meanwhile, other membrane materials will be investigated.

Although his research task primarily addresses the EVA element of Advanced Life Support, similar technologies have been demonstrated using dry air or nitrogen to sweep the membrane, instead of a vacuum, to cause water to evaporate. The evaporation of water, whether to vacuum or to a dry gas stream, will result in a cooling effect. Therefore this technology can be applied to a ground-based thermal control requirement.

A Novel Method For Air Revitalization-CO₂ Removal From Air By a Pulsating Device

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Funding:

UPN/Project Identification: 199-61-17-07

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 6

Post-Doctoral Associates: 0

FY 1997 Funding: \$65,994

Task Description:

This research involves moving large amounts of CO₂ from air and sending it in the form of a concentrated gas to a bioconverting environment such as plant photosynthesis. In this research, we will focus on the separation process and not on the bioconversion. The method is based on earlier established work in fluid dynamics oscillatory flows. The principles on which the method is founded depends on the tuning of oscillation frequencies to the time constant of diffusion of species. We will test the theory with experiments and optimize the removal process. Innovations include changing geometry and hydrodynamic conditions so as to effect a better separation between gases. This research concentrates on the separation mechanism and its optimization. The advantage to the space program is that a new and novel means of air revitalization will then become available with lower power requirements. Further, fundamental advances to the theory of gas dispersion by means of pulsating flows will be made.

There were two major achievements in the last period. The first was an experimental confirmation of the substantial enhancement in the mass transport due to oscillatory flow as well as a clear demonstration of the separation of species from air. The second achievement was a clear theoretical development that predicts this enhancement as well as the effect of geometry on separation. An important result of the theoretical development was that for the first time the mechanism of separation was revealed for species in gas as well as liquid phases.

The results of the work associated with this project have benefits in organic volatile gas removal from air in closed environments such as in future planetary exploration, submarine operation, etc. It will also have use in fine particle removal from gases as well as species separation in the liquid phase.

FY97 Publications, Presentations, and Other Accomplishments:

Beal, J., Zhang, X., and Narayanan, R. "The effect of annular geometry on enhanced mass transport in oscillatory flows." American Physical Society, Division of Fluid Mechanics (November, 1996).

Poplasky, M.S. Enhanced diffusion in the annular space between oscillating concentric cylinders. (Thesis) University of Florida (December, 1996).

Poplasky, M.S. and Narayanan, R. "Enhanced mass transport and separation of species using pulsatile flows." AIChE Annual Meeting, Chicago (November, 1996).

Testing an Algae-Based Air-Regeneration System Designed For Use in a Weightless Environment

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-61-17-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$13,326

Task Description:

The proposed project will investigate the feasibility of an air regeneration system based on subaerial algae growing on the surfaces of microporous ceramic tubes for space flight. The system proposed may present a number of advantages over bioregeneration systems using higher plants, particularly in terms of energy and space requirements. A simple prototype system has been designed and used to screen variety of unicellular algae. A number of basic questions concerning the growth of the algae on the ceramic tubes were addressed. Of particular interest was the rate of photosynthetic carbon uptake per tube as a function of the age of the tube, the photosynthetic photon flux, and the concentration of carbon dioxide.

At this point, preliminary screening has been completed for 6 strains of algae: *Chlorella fusca* var. *vacuolata* (UTEX 251), *Chlorella vulgaris* (UTEX 259), *Chlorococcum scabellum* (UTEX 1233), *Neosporangiococcum punctatum* (UTEX 786), *Gloeocapsa* sp. (VSU 104), and *Stichococcus* sp. (VSU 105). All grew strains tested grew well on the tubes. With periodic changes of the medium, reservoir growth could be maintained for at least one year. Carbon dioxide uptake increases with the age of the culture until the surface of the tube is covered with a dense coat of algae, at which point the rate of uptake stabilizes and then declines as contamination by bacteria and fungi worsens; the peak rate of uptake occurred between 150 and 180 days after inoculation. The maximum rate of CO₂ uptake measured was about 200 μmoles m⁻² min⁻¹, under a photosynthetic photon flux of 225 m⁻² s⁻¹ and a CO₂ concentration of 450-500 ppm. At this rate of uptake, 75 to 100 square meters of tubes would be required to meet the needs of each member of the crew. We are currently looking at ways to reduce the space requirements of the system by increasing the concentration of CO₂ and by changing the lighting to an LED-based system. We are also experimenting with thinner support systems for the algae.

This project is primarily concerned with the development of hardware for space travel. However, it is anticipated that the research will increase our understanding of the basic biology of subaerial algae. This ecological group is common in all terrestrial environments, forming visible growths on walls, rooftops, trees, and rocks. The ability of these organisms to exist and persist in environments lacking a permanent water supply is especially remarkable when one considers that unicellular organisms lack the usual protections against desiccation and are, therefore, subjected to repeated and prolonged periods of cryptobiosis in exposed locations. How they manage to survive, and even thrive, under these conditions remains an open question. The results of the present project should help to lay the foundation for further research into this area.

FY97 Publications, Presentations, and Other Accomplishments:

Beverly, J.T. "Preliminary identification of subaerial algae from southeastern Georgia and northern Florida." Presented at the Third Annual Symposium on Undergraduate Research, Valdosta State University (May 9, 1997).

Worthington, S.E. and Beverly, J.T. "Evaluating the growth of algae on ceramic tubes." Presented at the Third Annual Symposium on Undergraduate Research, Valdosta State University (May 9, 1997).

*Cryogenic PLSS Design Study***Principal Investigator:**

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Funding:

UPN/Project Identification: 199-71-17-03
 Initial Funding Date: 1997
 Students Funded Under Research: 0
 FY 1997 Funding: \$177,699

Solicitation: 96-OLMSA-01
 Expiration: 1998
 Post-Doctoral Associates: 0

Task Description:

Oceaneering Space Systems (OSS) has developed a liquid air backpack that is poised to revolutionize the self-contained breathing apparatus market for terrestrial workers. By providing breathing gas and cooling from the same source, it is smaller and lighter than its high pressure gas counterparts, and is simple, reliable, and inexpensive enough to compete with this 30 year old technology. The same technology can produce a similar revolution in Portable Life Support Systems (PLSS) for use in extra-vehicular activity in space.

This study proposes to design a liquid oxygen-based PLSS to a level at which its total life cycle cost can be compared with that of a high pressure oxygen system for use in Low Earth Orbit (LEO), as well as the lunar and Mars environments. OSS will review the requirements for a PLSS and develop figures of merit to compare the liquid oxygen PLSS with a conventional, high pressure oxygen PLSS. The design will be based on functional schematics developed in other NASA studies and on Oceaneering's expertise in cryogenic system design. It will include: liquid oxygen as a source for breathing gas and cooling; magnetic fluid management for phase separation; and an ejector for system ventilation. The methods for carbon dioxide and water removal, and supplemental cooling if required, will be traded off as part of the study. The study will produce: a detailed functional schematic; a three dimensional physical design in sufficient detail to assess maintainability and servicing; and an analysis of the relevant performance criteria, such as mass to orbit and deorbit, consumable usage, volume, on-board power and maintenance requirements. Because no liquid oxygen is to be available as a utility on Space Station, the study will also design on-orbit oxygen liquefaction equipment in enough detail to permit assessment of its life-cycle costs and impacts.

The life cycle costs of the current Shuttle Extravehicular Mobility Unit (EMU) and new upgraded EMUs will be researched as a basis for comparison. The figure of merit will be applied to both the liquid oxygen and compressed oxygen PLSS to substantiate the advantages of the approach.

This is a new project this year. Progress to date includes compilation of performance specifications for the US Space Shuttle EMU and the Russian EMU, and gathering EMU related mission requirements for space station,

lunar, and Mars missions. A preliminary schematic is being developed to be used for the PLSS design activities. Figure of merit indices are being developed to allow for life cycle cost comparisons of the different PLSS configurations.

This project is a design/comparison study of portable life support systems for Space Suit Systems for use in Low Earth Orbit, on the moon, and on Mars. The life cycle costs including development, use, and maintenance in space between conventional compressed gas and liquid oxygen-based systems will be compared. Benefits to Earth derive from having a lower cost, easier maintainable portable life support system on space missions. This will provide safer operations for astronauts, and allow additional experimental cargo to be flown in place of spare parts and tools needed for a more complex life support system. Spin-off technologies from NASA programs involving breathing and cooling systems used by firefighters and hazardous material workers provide an additional indirect Earth benefit.

Biochemical Capture and Removal of Carbon Dioxide

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UPN/Project Identification: 199-61-17-12

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$153,959

Task Description:

The principal objective of this proposal is to develop and test a new, highly efficient, light-weight, biochemical method to extract and capture CO₂ from respiratory gases. Our second objective is to develop a method for the on-orbit regeneration of the enzyme-matrix CO₂ extraction system to support long-term space missions.

We have enlarged our study of immobilization substrates. New studies focused on several types of glass surfaces. The data show retained activity (RA) of 17% for spherosil glass beads and somewhat lower RA for other types of glass. We believe these values, and that reported below, to be diffusionally limited. However, even this degree of activity is sufficient for CELSS applications.

These immobilization methods do not allow easy on-orbit regeneration of the bioreactor as promised with the chelated metal ligand approach. The Ni-NTA sepharose described last year are also not ideal for regeneration experiments. Thus, we reinvestigated the Ni-NTA binding using a product not previously available, Ni-NTA silica. Both the carboxy-His-6 tailed CA and the amino-His-6 tailed CA were immobilized to Ni-NTA silica. Each exhibited RA in excess of 12%. Both were resistant to repeated washing with buffer indicating that the immobilization was stable. However, they could be removed from the chelated metal immobilization site by the competitive agent imidazole. The Ni-NTA was then available for a next round of immobilization. This observation represents the completion of a key specific aim and will allow on-orbit regeneration of the bioreactor by means of only a few grams of enzyme.

During this year we began studying longevity of the enzyme. While lyophilized enzyme has very long shelf life (years) we have now demonstrated undiminished activity for periods in excess of 30 days for dissolved forms the enzyme. Studies will continue this year to determine if and when the enzyme activity declines.

We studied several different types of membrane systems - thin liquid film, proteic membranes and teflon membranes - as examples of enzyme-based facilitated transport membranes systems. Each of these showed a facilitation of CO₂ transport with the improvement ranging up to 15-fold and selectivity factors of several hundred-fold over oxygen. Initial data show the membranes to be resistant to fouling. The membranes were progressively more efficient as the CO₂ partial pressure decreased. They are particularly efficient at CO₂ values of 0.1%. This result suggests that we should be able to achieve near Earth ambient CO₂ levels for the crew. These data also satisfy a key specific aim.

Future work will see the production of a scaled-up bioreactor for systems testing.

One possible medical application is development of closed cycle anesthesia machines for gaseous anesthetics. However, carbon dioxide capture followed by concentration or sequestration has many terrestrial applications. Examples of closed environmental life support applications include hazardous materials handling, mine safety, aircraft and submarines, scuba diving, and fire rescue.

A longer term and potentially more significant terrestrial impact of this project will be in scale up of the system as a means of capturing carbon dioxide currently released into the atmosphere, thus helping to ameliorate greenhouse gases. Point sources account for more than one-third of all of the carbon dioxide produced. However, the small size of this system may also allow its use with mobile CO₂ sources. Economically attractive availability of CO₂ is expected to result in development of additional new technologies for applying the available CO₂; examples include enhanced agriculture and aquaculture.

FY97 Publications, Presentations, and Other Accomplishments:

Trachtenberg, M.C. NASA ALS meeting, L.B. Johnson Space Center, Houston, TX (October 9 - 11, 1996).

Trachtenberg, M.C., Tu, C.K., Landers, R.A., Willson, R.C., McGregor, M.L., Laipis, P.J., Kennedy, J.F., Paterson, M., Silverman, D.N., Thomas, D., and Rudolph, F.B. "Carbon dioxide transport by proteic and facilitated transport membranes." *Life Supp. Biosphere Sci.* (In Press).

CELSS Crop Simulations for Systems Engineering and Productivity Optimization

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-61-17-23

Solicitation: not available

Initial Funding Date: 1994

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$119,998

Task Description:

This research continues work on a progressive series of mathematical models for the CELSS hydroponic crops. Researchers in the CELSS Program are investigating the growth and development of the candidate crops in a variety of controlled environments. A central objective of this research is to use these experimental findings for (a) systematizing crop data into engineering models that can be integrated into system-level considerations, and (b) analyzing and predicting optimal conditions for new generation of experiments.

The approach continues the strong collaboration established over previous years with Bruce Bugbee of Utah State University, and also with Ray Wheeler and Gary Stutte of Kennedy Space Center. Benefits to the overall program derive from a modeler working with the experimenters, asking questions, formulating and reformulating models, and publishing collaborative papers that organize the data into a common modeling framework. To address the most important scientific issues about the CELSS crops, the key modeling inputs are the gas exchange data from the above institutions. Gas exchange data are also now becoming available from Ames Research Center and Johnson Space Center.

These general tasks will be specifically accomplished in two major research arenas. First, development of the energy cascade as a modeling strategy that examines the components of crop growth as a sequence of conversion efficiencies. These components require ongoing analysis and prediction because they are relevant to the CELSS Program as fundamental processes of crop growth, and because they are relevant to the CELSS engineers for inclusion in general system design. Second, a new initiative will employ the relatively elaborate field crop models (Ceres-wheat, Soygro, Substor-potato, etc.). Based on the principal investigator's experience with simpler models for the CELSS crops over the years and collaborations with the crop researchers, these field models will be modified and used as modeling tools to predict experiments to increase yield and optimize total life cycle productivity with phasic controls.

Progress has been made in studying: (1) phasic control of productivity, by which specifying a sequence of environmental conditions during a crop's life cycle may increase a chosen measure of productivity, such as grams of food per square meter per day; (2) the effects of the high amount of diffuse light in growth chambers on productivity.

Regarding phasic control, results from our modified Ceres-Wheat model indicate that grain yield might be increased by 15% to 20% over the best yield at constant temperature, using five different temperatures during the life cycle (Volk et al., 1997). We also have developed a modified Cropgro model (Cavazzoni, 1997; Cavazzoni et al., 1997). Soy proves to be an entirely different creature. Restrictions on its productive temperature range and the "flatness" of several of its key variables with respect to temperature results in very little enhancement with phasic control of temperature, explored with the model. But interestingly, soy offers opportunities for phasic control by daylength. According to the model, a slight increase in daylength during the second half of the life cycle will increase daily average productivity by 10%. These results for phasic control for wheat and soy are encouraging, and we would hope that chamber experiments begin to test these predictions.

Regarding the issue of light quality in the chambers, we have assessed the importance of diffuse light using a ten LAI layer canopy photosynthesis model, with "sunlit" and "shaded" leaf classes per layer, and a three point Gaussian integration of photosynthesis over leaf angle classes for "sunlit" leaves. For modeling purposes, we define a direction for direct light in order to calculate a direct light extinction coefficient. Comparisons are made of different leaf angle distributions, with diffuse light extinction coefficients being either constant or varying with canopy depth. Based on an examination of model sensitivity to diffuse light, the leaf angle distribution and different light extinction coefficients (for direct and diffuse light) are important variables when modeling crop response to diffuse light. These results, which are being written up, should help in the design of advanced lighting conditions for maximum productivity.

Given that this work is advancing the models that are currently being applied to agricultural crops on Earth, is it reasonable to expect that this research will help understand and predict the potential changes in agriculture that might occur from global change, in particular the responses of crops to changes in temperature and carbon dioxide. For example, based on how we have modeled the experimental results from the NASA wheat, we have been able to apply the modified Ceres-Wheat model to explore yield shifts caused by simultaneous warming and higher carbon dioxide levels, both possibilities on a near-future Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Cavazzoni, J. Dynamic modeling of crop productivity on a dual scale: Simulating soybean growth under controlled environments; assessing implications of increased crop yield on a global scale. (Dissertation) New York University (February, 1997).

Cavazzoni, J., Volk, T., and Stutte, G. "A modified Cropgro model for simulating soybean growth in controlled environments." *Life Supp. Biosphere Sci.* (In Press).

Tubiello, F.N., Volk, T., and Bugbee, B. "Diffuse light and wheat radiation-use efficiency in a controlled environment." *Life Supp. Biosphere Sci.* (In Press).

Volk, T. "CELSS crop simulations for systems engineering and productivity optimization." Advanced Life Support Requirements Workshop, NASA Johnson Space Center, Houston (October 28 - 30, 1996).

Volk, T., Bugbee, B., and Tubiello, F. "Testing the Ceres-Wheat model with growth chamber data under elevated CO₂." American Society of Agronomy Annual Meeting, Cincinnati (November, 1996).

Volk, T., Bugbee, B., and Tubiello, F.N. "Phasic temperature control appraised with the Ceres-wheat model." *Life Supp. Biosphere Sci.* (In Press).

Performance Assessment Using Dynamic Simulation and Human Factors Analysis in IVA and EVA

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Funding:

UPN/Project Identification: 199-06-17-06
Initial Funding Date: 1997
Students Funded Under Research: 3
FY 1997 Funding: \$200,000

Solicitation: 96-OLMSA-01
Expiration: 2000
Post-Doctoral Associates: 0

Task Description:

The overall project goal is to produce computerized human performance measurement, evaluation, and modeling techniques for IVA and EVA activities. Performance modeling is needed to assess task feasibility and human workload for routine, novel, or unexpected tasks in space. Crewmember tasks include EVA suited as well as unsuited activities. The target is to make tangible progress toward non-invasive video motion capture of astronaut activity for real-time task analysis, workload monitoring, and safety assessment based on computer modeling and computer vision techniques. Performance measures encompass kinematic (range of motion), kinetic (joint torques or end effector forces related to strength), and workload analysis (metabolic load, stamina, and fatigue). Performance models will be based on both empirical and simulation data: empirical or existing data from NASA studies and sources, and simulation data from physics- and control-based computer human motion simulations. Control regimes may vary according to the needs of different task components. Gaps in empirical data, such as detailed EVA suit characteristics, will be noted for possible NASA data acquisition experiments. The developed software will permit modular insertion of suit and crewmember data, and the convenient substitution of various performance models. Applying biomechanical modeling and physics-based dynamic simulation will establish analytic and predictive measures for IVA and EVA space human factors. Such simulations will therefore provide direct inputs to force feedback devices for VR-based training. This project will build on existing dynamic, anthropometric, and kinematic models for human motion and EVA activities developed by the investigators at the Massachusetts Institute of Technology and the University of Pennsylvania.

We are designing an integrated IVA/EVA modeling system that is based on data acquired from previous astronaut activities and will use modern sensing techniques to aid in various space related tasks. This project will build on existing dynamic and kinematic models for human motion and EVA activities developed by the investigators.

To date, our research has focused on the development of efficient methods for the forward dynamic simulation and control of virtual humans. We have developed a physics-based system for the guided animation of virtual humans and other articulated figures. Based on an efficient recursive dynamics simulator, we have developed a robust feedback control scheme and a fast two-stage collision response algorithm. A user of our system provides

kinematic trajectories for those degrees of freedom (DOFs) of the figure they want direct control over. The output motion is generated using forward dynamics. The specified motion trajectories are the input to a control system which computes the forces and torques that should be exerted to achieve the desired motion. The dynamic controllers, designed based on the Model Reference Adaptive Control paradigm, continuously self-adjust for optimal performance in trajectory following. Moreover, the user is given a handle on the type and speed of reaction of the figure's controlled DOFs to sudden changes in their desired motion. This system should provide a platform for generating and studying realistic user controlled motion of articulated objects at interactive rates. We require minimal user involvement in specifying non-intuitive parameters. Actual IVA/EVA human performance may be used to determine control parameters in the next stage of work.

We have also developed a novel method for modeling dynamic systems with open- and closed-loop dynamics such as ladder climbing in simulated virtual worlds. This work is a generalization of our previous research involving the control of dynamic systems with open loops. To model closed-loop dynamics, we use the Lagrange multiplier method which results in a system of differential algebraic equations (DAE). We also use the Lagrange method for the dynamic formulation of the forward dynamics. To simulate a human performing a task where closed loops are involved, the input to the algorithm is a given forward velocity, step length, step frequency, and a chosen gait. The algorithm then determines some kinematic aspects of the motion pattern (e.g., the timing for the stance phase, the double support and swing phases in case of ladder climbing). These positions are fed into the dynamic formulation of our system to determine the motion of the human between the initial and final positions. We employ an iterative numerical analysis technique which uses the Newton-Raphson method to find the vector of joint torques that drives the dynamic system from the initial position to the final position. The stability problem during the iterative numerical integration is addressed by applying the Baumgarte stabilization method.

Although ladder climbing is not a typical microgravity activity, the mathematical and software basis for managing multiple points of contact under dynamic propulsion conditions is a crucial aspect of microgravity locomotion. These techniques are almost ready to be extended to that domain. Thus we are currently working on extending the above framework to incorporate optimal control methods within a recursive dynamics formulation. This extension will allow the computer-based planning of astronaut motion in space under the minimization of certain criteria (e.g., exertion of minimal torque by the astronaut during a desired set of motions).

The overall project goal is to produce a realistic human performance modeling technique tailored to IVA and EVA activities. This technique would be used to measure and evaluate task feasibility as well as develop new methods of task assignment in space related activities. This will enhance ground-based IVA and EVA procedure design as well as predict payload handling difficulties. Moreover, the potential predictive power of workload models will enhance our computational understanding of other Earth-bound activities.

Power Assisted Space Suit Joint

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Funding:

UPN/Project Identification: 199-06-17-04

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 199,862

Task Description:

The continuous development of Extravehicular Activity (EVA) spacesuit gloves has led to an effective solution for performing extravehicular activity to date. Some limitations of the EVA gloves have been noted that potentially affect productivity in the form of limited dexterity and high torque joints that accelerate the onset of fatigue (Lapham, 1994). The limitations of the current gloves in conjunction with the fact that the mission envelope is currently expanding via task complexity, frequency, and duration, indicate the need for further development of spacesuit gloves.

The spacesuit gloves have evolved over years of development. The emphasis of this development has been on the advancement of a passive restraint and mobility system. The development of advanced materials and processes has led to steps forward in this technology but no quantum leap has been realized. Technology is now available to make a leap in performance capability which will improve astronaut effectiveness during EVA. This increased performance will also reduce mission costs by reducing crew fatigue and increasing mobility, thus increasing task speed and mission duration.

By utilizing technology from the current spacesuit glove in conjunction with state-of-the-art robotics technology, a power-assisted spacesuit glove joint can be created to greatly improve glove performance. The power-assisted joint will only provide enough force to offset the resistance of the pressurized glove joint itself and will not provide strength augmentation to the hand. In this approach, nude body performance will be approached via tuning of the power assist system. Of principal interest will be the improvements in the areas of dexterity and fatigue, but the technology will also address factors involving tactility, range of motion, and comfort. By effectively applying these technologies a synergism will be created that will allow the EVA crewmember to maintain a "hands on" approach that will enhance mission effectiveness by keeping the most important element of the EVA, the crew member, at the worksite.

Throughout FY97, the metacarpophalangeal (MCP) joint design was refined to reduce torque and hysteresis in the joint. Additionally, the control algorithms and actuation systems were refined to minimize power requirements and to improve smoothness of the MCP through its full range of motion.

The refinement process culminated in a working prototype which was demonstrated early in the year. During this demonstration, six different test subjects were fitted with EMG sensors, fitted into the glove, and performed MCP joint activities designed to exercise the MCP joint. Results clearly showed that fatigue was significantly reduced (30 - 40%), and range of motion of the MCP joint was substantially improved (~440%).

The successful functional demonstration clearly revealed that the proposed concept is sound in principle, and will offer substantial fatigue and range of motion performance improvements for future EVA work.

Ongoing work includes further refinement of the entire control, actuation, and MCP joint systems. Another functional demonstration is scheduled for late December of 1997.

Due to the high success seen in Phases I and II of this NRA, planning for Phase III of the program is in process, and is highly recommended for this successful technology.

The current research into power assisted space suit gloves, specifically the MCP joint, is meant to first demonstrate this technology in one area of immediate need. In the future, this technology could be applied to other spacesuit joints such as the glove's carpometacarpal joint, wrist joint, shoulder joint, and hip joint. Application of the technology in these areas would reduce user fatigue, increase mobility, and even augment strength. These aspects of spacesuit design will be imperative for future Lunar and Martian exploration missions.

In addition to the above mentioned space applications, the power assisted glove technology could be beneficial in several other industries, including the medical and commercial entertainment industries. The power assist system, coupled with the appropriate sensors, could be used to facilitate movement of prosthetic devices or appendages of persons with loss of mobility. Virtual reality technology could benefit by using force feedback suits to enhance sensory feedback in the virtual reality environment. In the ergonomics and rehabilitation fields, the power assist glove could be used to perform ergonomic evaluations of persons suffering from compromised mobility and reduced strength in their hands or limbs. In addition, the power assisted glove could be modified to provide force feedback, thereby acting as a rehabilitation device.

FY97 Publications, Presentations, and Other Accomplishments:

Sorenson, E.A., Sanner, R.M., and Ranniger, C.U. "Experimental testing of a power-assisted space suit glove joint." Space Systems Laboratory (1997).

Sorenson, E.A., Sanner, R.M., Howard, R.D., Akin, D.L., Cadogan, D.P., and Lingo, R.W. "Development of a power-assisted space suit glove joint." Space Systems Laboratory and ILC Dover, Inc. (1997).

Performance in Haptic Virtual Environments with Visual Supplement

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Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$111,000

Responsible NASA Center: Ames Research Center

Task Description:

The goal of this work is to determine human factors guidelines for effective haptic (force reflecting) manual interfaces for multisensory virtual simulator and teleoperation displays. The two major aspects of this applied research and development project are: 1) the design and implementation of an innovative, high performance three degree-of-freedom (dof) force reflecting manual interface for use with our laboratory's virtual visual display; and 2) examination of human perception and manual task performance, respectively, through psychophysical discrimination and manual target acquisition experiments with the combined haptic-visual virtual environment (VE) research testbed. Application areas for improved force displays include simulator and teleoperator interfaces for design prototyping, training, and maintenance in spacecraft assembly, telepresence for planetary exploration, advanced scientific data visualization, and on-orbit physiological and psychophysical research.

During FY 1997, we completed fabrication and assembly of the three dof force-reflecting haptic interface design described in the previous year's report. The final version of the prototype joystick maintains the original workspace specifications: mechanical limit-stops installed to prevent excessive motion by the linkage allow the linkage to reach a 30 cm wide by 15 cm high by 55 cm deep singularity-free work volume while avoiding internal interference between its mechanical links. Quantification of output force ranges and simulated impedances achievable with this device is underway.

An additional feature designed and installed during 1997 is a self-contained spherical handgrip which serves as a passive (i.e., non-actuated) three dof "wrist" between the operator's hand and the miniature six axis force transducer at the distal end of the three dof actuated interface mechanical linkage. This wrist allows the handgrip to maintain a constant orientation relative to the machine's base regardless of the actuated linkage's configuration. Furthermore, the handgrip in combination with the wrist mechanism is mass-balanced such that it applies a fixed magnitude force (due to its weight) at the transducer regardless of handle orientation and joystick linkage configuration.

Thus far, we have developed a series of fundamental real-time controllers such as isotropic stiffness fields and unidirectional constraints (i.e., "virtual walls") on a Pentium-II PC that are presented by the three dof haptic

interface. In general, these simple haptic wall surfaces serve as the basic elements from which virtual haptic objects are constructed (just as surfaces and polygons would in a graphics environment).

Our initial controllers build upon PID position control of the linkage in actuator joint angle space as well as in Cartesian endpoint space. In fact, we have employed these PID routines to make the joystick hardware behave like a conventional programmable robot manipulator, capable of following a sequence of endpoint spatial trajectory commands. We have also demonstrated computer control to actively balance the haptic interface's linkage component masses. This forward path compensator, by canceling the configuration-dependent gravity forces acting on the ten-link joystick mechanism, serves to validate the mass distribution models we derived for the device.

The control software is written in C++ and is modular—for example, basic elements such as the PID feedback control, task scheduling and joint space to endpoint kinematic mappings (e.g., forward and inverse Jacobians) can be reused. Without yet having optimized software timing characteristics, the fundamental controllers described above have attained update rates in excess of 1-5 KHz.

Concurrent with design and fabrication of the haptic interface, we developed, with shared support from Code UL Task 199061238, advanced real-time software techniques and protocols for spatially calibrated, very low latency (21-26 msec) and high update (58-60 Hz) response of the visual component of the VE system hosted by our main laboratory graphics computer. The high speed communication and spatial correction protocols that we have implemented for use with commercially available VE motion sensors enable the requisite dynamic accuracy for perceptually stable head-tracked visual display and will be used for integration of the force-reflecting handcontroller into the combined visual-haptic testbed.

This task has two major components. The first is the design and construction of an innovative force reflecting manual interface capable of very high fidelity haptic interaction and information display. The second component is human factors research in virtual environments using this new haptic interface, both alone and in conjunction with a coordinated visual display.

The goal of the human factors work is the development of guidelines and specifications for effective computer controlled haptic information presentation, for haptic interaction in isolation and when combined haptic-visual display is available. Because the study of human haptic interaction and perception of mechanical environments and especially of digitally controlled (i.e., computer-generated), mechanical (i.e., haptic) simulation is a new area of research, results of this work would benefit the development of effective haptic interface and virtual environment displays in many fields of endeavor.

Computer-modulated and generated haptic and visual displays for virtual environments will enhance individual and crew performance on Earth and in space, in aspects that involve simulation, including training and rapid design prototyping for manual interaction with hand tools and control panels, scientific data visualization, and on-line interaction for remote manipulation.

Medicine, an activity in which precision manual interaction plays a very significant role, is one specific area of application for this technology. As such, haptics researchers and equipment developers have been giving much attention to the problems of training, planning, and execution for surgical procedures in nearly all parts of the human body.

A plausible space medicine application would employ a computer-controlled haptic interface capable of generating specific forces or mechanical dynamics to compensate for strength and muscle changes caused by prolonged exposure to microgravity, to counteract the limitations of space flight tools, gloves, and suits, or, simply to emulate on a hand or other body part normal gravity forces that are otherwise significantly altered by space flight. Similarly, on Earth, this haptic interface technology can be used to compensate for abnormal limb motion and force characteristics in people impaired by neuromuscular disorders.

FY97 Publications, Presentations, and Other Accomplishments:

Adelstein, B.D., Ho, P., and Kazerooni, H. "Kinematic design of a three degree of freedom parallel hand controller mechanism." Proceedings, Dynamic Systems and Control, DSC-58, American Society of Mechanical Engineers, New York, pp. 539-546 (1996).

Ho, P.P.-M. Kinematic analysis and prototype design of a 3-Degree-of-Freedom Hand Controller. (Thesis) Department of Mechanical Engineering, University of California, Berkeley (December, 1996).

Visual Performance and Fatigue in See-Through Head-Mounted Displays

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UPN/Project Identification: 199-06-12-38

Solicitation: 93-OLMSA-07

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Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$205,000

Joint Agency Participation: ARPA (TRP)

Responsible NASA Center: Ames Research Center

Task Description:

An opto-electronic test bed, an electronic haploscope, will be used for human factors testing of hardware and software to guide development and evaluation of head-mounted, see-through displays. This kind of display is being developed by the U.S. commercial aircraft industry to assist aircraft assembly. It also may be used for visualization and control of near (< 1 m) virtual images in vehicle and equipment maintenance displays as well as in head mounted displays for teleoperations and telerobotics and for on-orbit physiological and psychophysical investigations.

The head-mounted see-through displays developed for this task have continued to be used to examine the cause of errors in human observers' depth judgments to computer generated virtual objects. We have also studied the consequences of monocular, biocular, or stereoscopic viewing on the accuracy of these depth judgments and the subjective viewing discomfort while making them for extended periods of time. The displays used in these studies have been proposed to dramatically increase productivity in several manufacturing environments and to be unique stimulus presentation formats for scientific research. Both of these goals have been advanced by our research. Field trials for some aircraft manufacturing applications took place in mid-1997.

The reduction of measured full system rendering delay for the presentation of head-stabilized stereoscopic virtual objects has, however, remained a significant problem for potential use of these displays. Recent development under this project has reduced the full system delay from 65 msec with 20 Hz updates to approximately 25 msec and near 60 Hz. This performance is currently the best dynamic response of a rendering system like this in the world. These accomplishments have been reported in refereed proceedings papers and as publications in refereed journals. Studies of the accuracy with which monocular systems can display objects in depth using head motion cues have been completed and suggest that further reduction in system latency should significantly improve performance. Position sensor performance has additionally been improved through implementation of a spatial distortion correction algorithm using a tetrahedral spatial decomposition scheme. This technique combined with interpolation techniques for position and orientation have reduced the static position error to millimeters in most of the volume used for experiments.

Since there is an evident need to investigate improved dynamic response of our virtual object display systems, our laboratory has been working continually to optimize our hardware and software so as to maximize frame rate and minimize latency. As documented in publications, through approximately 1.5 man-years of development, we have substantially improved dynamic response of the Skywriter-based hardware. To our knowledge, our latency performance with the current Skywriter has the lowest latency for any type of SGI graphics equipment anywhere, even systems with more powerful Reality Engines 2 or Infinite Reality graphics. This performance gives us a unique ability to study new phenomena which only can be seen with low latency virtual object rendering. We accordingly can look into the future when faster interactivity with virtual objects is more widely available.

In 1997, the third version of the electronic haploscope was delivered to NASA. It provides a 30-40 degree monocular field of view with 40 to 100% variable overlap with visual resolution equivalent to the last version of the haploscope, but now the display can present full color images at full 640 X 480 resolution. This display will be used in future experiments on the perceptual stability of virtual objects planned for the next three years.

Virtual environment displays may provide a new communications medium for spatial information. The research conducted on this project is directed to improving the dynamic fidelity of these displays and investigating phenomena that affect their application to a wide variety of practical problems. These displays can be used, for example, to view simulations of industrial robotics, and to assist programming robots on assembly lines, visualizing CAD/CAM drawings and computer graphics based preassembly testing as done with the Boeing 777. They are natural media for viewing the outputs of rapid prototyping systems for manufacturing and in see-through versions as information displays for mechanical assembly, equipment maintenance, and component testing. In fact, projects demonstrating these applications are currently underway at Boeing Computer Services in Bellevue, Washington and McClellan AFB north of Sacramento, California. At the latter site, head-mounted displays for wearable computers have been shown to dramatically increase productivity of workers examining KC135 fuselages for cracks in their skin.

Virtual environment displays can be used to present visual, acoustic, or haptic stimuli used in psychological or physiological investigations and thus can help advance scientific research. In fact, the virtual display format makes possible the presentation of patterns of sensory information that are not physically realizable and can give researchers control over sensory stimuli to be used in their experiments.

Virtual displays have more practical applications as new human interfaces for endoscopic or laparoscopic surgery as well as tools of surgical training and the remote consultation associated with telemedicine. Thus, the displays are also useful for instruction (e.g., medical students can use them to be given a very concrete view of what they would see if they were to execute the task they are studying). Similar applications exist for other fields, including 3-D data visualization, geographic information systems, entertainment, and video games. More detailed discussion of the widespread applications of virtual environments can be found in the general reference articles cited below.

Virtual object displays have recently been suggested as aids to maintain visual contact between air traffic controllers and taxiing aircraft during low visibility conditions. Information as to how to accurately present virtual objects without significant visual fatigue that will be developed by the current project should contribute to the design of head-mounted visual display aids for the air traffic control tower.

FY97 Publications, Presentations, and Other Accomplishments:

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Ellis, S.R., Begault, D.R., and Wenzel, E.M. "Virtual environments as human-computer interfaces" in "Handbook of Human-Computer Interaction." Edited by: Helander, M., Landauer, T., and Prabhu, P. pp 163-228 (1997).

Ellis, S.R., Bréant, F., Menges, B.M., and Adelstein, B.D. "Viewing difficulties with head-mounted aids for mechanical assembly." Presented at the 5th IEEE International Conference on Emerging Technologies and Factory Automation, Kauai, HI (November 18 - 21, 1996).

Ellis, S.R., Bréant, F., Menges, B.M., Jacoby, R.H., and Adelstein, B.D. "Factors influencing operator interaction with virtual objects viewed via head-mounted see-through displays: Viewing conditions and rendering latency." *Proceedings of VRAIS '97*, Albuquerque, NM. IEEE, pp. 138-145 (1997).

Ellis, S.R., Bréant, F., Menges, B.M., Jacoby, R.H., and Adelstein, B.D. "Operator interaction with virtual objects: effects of system latency." *Proceedings of HCI '97 International*. San Francisco, CA, pp. 973-976 (1997).

Ellis, S.R., Dorighi, N.S., Menges, B.M., Adelstein, B.D., and Jacoby, R.H. "In search of equivalence classes in subjective scales of reality." *Proceedings of HCI97 International*. San Francisco, CA, pp. 863-876 (1997).

Ellis, S.R., Menges, B.M., Jacoby, R.H., Adelstein, B.D., and McCandless, J.W. "Influence of head motion on the judged distance of monocularly presented virtual objects." *Proceeding of the Human Factors and Ergonomics Society 41st Annual Meeting*, Albuquerque, NM, HFES, Santa Monica CA, pp. 1234-1238 (1997).

Rogers, W.A. "Effects of age on the judged distance to virtual objects in the near visual field" in "Designing for an Aging Population: Ten years of Human Factors/Ergonomics Research." *Human Factors and Ergonomics Society*, Santa Monica, CA, pp 15-19 (1997).

Perceptually-Tuned Visual Simulation

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Funding:

UPN/Project Identification: 199-06-12-05
Initial Funding Date: 1995
Students Funded Under Research: 4
FY 1997 Funding: \$157,500

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 1

Responsible NASA Center: Ames Research Center

Task Description:

Human factors engineering is required to improve the quality of visual displays in space systems. Advanced computer generated imagery (CGI) systems are used to create compelling visual displays for navigation/control systems, vehicle/system simulation, telerobotics, and scientific visualization applications. The quality of these displays can impact the safety and productivity of space and ground-based operations. Inevitably, the realism of these displays is constrained by limitations in CGI hardware and software, especially if images need to be generated in real-time. Despite rapid advances in image generation technology, human operators desire more realistic, higher-fidelity displays; it is likely that such a demand for improved fidelity will continue for the foreseeable future.

We have conducted a program of research examining techniques aimed at reducing the computation cost required to achieve a desired level of image quality and frame rate. All of these techniques exploit principles of visual processing to reduce computational load. The first technique exploits properties of visual fusion to create images having more apparent resolution than is actually rendered. The second technique automates the ongoing trade-off between image quality and frame-rate via a system that degrades aspects of the scene based upon what is known to be most important to the visual system. Finally, the third set of techniques will develop more efficient algorithms for rendering motions in three-dimensions based upon principles of visual motion processing. This research requires a multidisciplinary approach and will involve a collaboration among research scientists at the NASA Ames Research Center, professors in Computer Science and Psychology/Biomedical Engineering at the University of Virginia, and designers and engineers at Silicon Graphics, Inc. and other industry sites.

Several techniques for graphical rendering have been developed and validated. The most promising of these is the differential resolution stereo technique, which exploits the fact that the visual system is able to recover disparity-specified depth from the low spatial frequency components of a stereo pair, and fuse the high resolution features present in only one of the images over the resulting percept. We have also validated techniques for modulating level of detail transformation, and evaluated observer sensitivity to "billboarded" objects.

New question domains have asserted themselves, notably the need to develop a reasonable taxonomy of levels of immersion in spatial displays (and map these levels into task requirements), and how immersive displays can be computationally simplified with minimal impact on the user. These emergent questions form the basis of our new research proposal (submitted for FY98-00 support).

Virtually all of the rendering techniques developed in this program will benefit Earth-based simulation and visualization systems in addition to those systems mounted onboard manned missions. All computer graphics systems are mounted with some constraints, be they cost, space, power, and/or reliability. Our techniques, which reduce the required computational complexity for a desired level of visual fidelity, can be exploited to reduce the hardware and/or software necessary for a system to perform at a given, required level of realism.

FY97 Publications, Presentations, and Other Accomplishments:

Bhalla, M. and Proffitt, D.R. "Geographical slant perception: Dissociation and coordination between explicit awareness and visually guided actions" in "Dissociation but Interaction between Nonconscious and Conscious Processing." Edited by: Rossetti, Y. and Revonsuo, A. Amsterdam: John Benjamins (In Press).

Creem, S.H. and Proffitt, D.R. "Separate memories for visual guidance and explicit awareness: The roles of time and place" in "Stratification of Consciousness and Cognition." Edited by: Challis, B.H. and Velichkovsky, B.M. Amsterdam: John Benjamins (In Press).

Creem, S.H. and Proffitt, D.R. "Two memories for geographical slant: Separation and interdependence of action and awareness." *Psychonomic Bull. & Rev.* (In Press).

Creem, S.H., Wade, M.M., and Proffitt, D.R. "Visual perception of egocentric distance." (Poster) Presented at the 38th Annual Meeting of the Association for Research in Vision and Ophthalmology (May, 1997).

Creem, S.H., Wraga, M., and Proffitt, D.R. "The significance of imagined viewer rotations" in "Studies in Perception and Action IV." Edited by: Schmuckler, M.A. and Kennedy, J.M. Hillsdale, NJ: Lawrence Erlbaum Associates., pp 35-38 (1997).

Domini, F., Caudek, C., and Proffitt, D.R. "Misperceptions of angular velocities influence the perception of rigidity in the kinetic depth effect." *J. Exp. Psych.: Human Perception and Performance*, 23, 1111-1129.

Durgin, F.H. and Proffitt, D.R. "Visual learning in the perception of texture: Simple and contingent aftereffects of texture density." *Spatial Vision*, 9, 423-474 (1996).

Hecht, H., Kaiser, M.K., and Banks, M.S. "Gravitational acceleration as a cue for absolute size and distance." *Perception & Psychophysics*, 58, 1066-1075 (1996).

Hecht, H., Kaiser, M.K., Savelsbergh, G.J.P., and van der Kamp, J. "The effect of time-sampling on temporal range judgments in real and simulated environments." Presented at the 37th Annual Meeting of the Psychonomic Society (November, 1996).

Kaiser, M.K. "The perception of dynamical constancies" in "Perceptual Constancy." Edited by: Walsh, V. and Kulikowski, J. Cambridge: Cambridge University Press (In Press).

Kaiser, M.K. "High power graphic computers for visual simulation: A real-time rendering revolution." *Behav. Res. Meth., Instr., & Comp.*, 28, 233-238 (1996).

Kaiser, M.K. "Moving through time: The utility of a temporal metric for vehicle control." Presented at the Ninth International Conference on Perception and Action (July, 1997).

Kaiser, M.K. and Montegut, M.J. "Of red planets and Indigo computers: Mars database visualization as an example of platform downsizing." *Behav. Res. Meth., Instr., & Comp.*, 29, 48-53 (1997).

Pausch, R., Proffitt, D.R., and Williams, G. "Quantifying immersion in virtual reality." Presented at the ACM SIGGRAPH Convention (August, 1997).

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Proffitt, D.R. "Naive physics" in "MIT Encyclopedia of Cognitive Science." Cambridge, MA: MIT Press (In Press).

Proffitt, D.R. "Perception: Ecological versus inferential approaches" in "The Concept of Cognition." Edited by: Sternberg, R.J. Cambridge: MIT Press (In Press).

Proffitt, D.R. and Kaiser, M.K. "The internalization of perceptual processing constraints" in "Handbook of Perception and Cognition, 2nd Edition: Perception and Cognition at Century's End." Edited by: Hochberg, J. San Diego: Academic Press (In Press).

Proffitt, D.R. and Kaiser, M.K. "Hi-Lo stereo fusion." *Visual Proceedings of SIGGRAPH'96*, pp. 146 (1996).

Wade, M.M., Wraga, M., Williams, G., and Proffitt, D.R. "Eye height-scaling of virtual scenes." Presented at the 38th Annual Meeting of the Association for Research in Vision and Ophthalmology (May, 1997).

Wraga, M., Creem, S.H., and Proffitt, D.R. "Spatial frames of reference in perceiving and acting" in "Spatial Navigation in People, Animals, and Machines." Edited by: McBeath, M. NY: Sage (In Press).

Yang, T.L., Wade, M.M., and Proffitt, D.R. "Overestimation of heights is greater for real objects than for objects in pictures." (Poster) Presented at the 38th Meeting of the Association for Research in Vision and Ophthalmology (May, 1997).

An EVA Strength and Reach Model

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UPN/Project Identification: 199-06-11-46

Solicitation: 93-OLMSA-07

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$60,000

Responsible NASA Center: Johnson Space Center

Task Description:

One of the goals in human modeling at the Graphics Research and Analysis Facility (GRAF) at NASA JSC is to create a task-oriented Extravehicular Mobility Unit (EMU) -suited human figure simulation which emulates the physical characteristics of the actual EMU-suited human counterpart as closely as possible. EMU simulations are commonly used at the GRAF for Human Factors reach and fit analyses. Nevertheless, a comprehensive, validated model for the EMU does not exist. Important components of such a model would include accurate reaching, strength capability, and fatigue parameters. We propose a project which will build a model of the EMU-suited crew member encompassing reach and maximum strength capabilities. Mission planners can use the modeling system and view animations and visualizations of the various parameters, such as overall motion, reach, and strength to streamline timing, duration, task arrangement and personnel for overall efficiency of the Extra Vehicular Activity (EVA) tasks. With previous NASA research funding, GRAF has incorporated an unsuited strength prediction capability into a computer model of the human arm. This model is based on empirically collected isolated joint strength data. Initial validation of the strength model has been successful for a multi-jointed arm motion (ratchet wrenching). To extend this model to the EMU-suited human will require collecting EMU-suited maximal strength and range of motion data for all the major suit joints. Accurate suit dimension measurements, corresponding anthropometry and joint limit data will be the basis for modeling the suit and human inside. The strength data will be processed into a compact format and embedded into the EMU model using the techniques developed with the unsuited strength model. Motion analysis data along with collected multi-joint motion torque data will be used to validate the EMU kinematic and strength model.

EMU-suited maximum strength and range of motion data for all the major suit joints has been collected. The strength data was processed into a compact format and embedded into the EMU model using the techniques developed with the unsuited strength model. A volumetrically accurate EMU suit model has validated for the basic and enhanced versions of the suit. A suit sizing model has also been developed which, when used in conjunction with an anthropometric spreadsheet, will tabulate and build the suit sizing rings required for accurate fit of the enhanced suit for any particular individual or population of individuals (Astronaut Candidate Database). In addition, an anthropometry prediction model has been developed which uses statistical techniques to estimate measurements for missing or erroneous data. This tool has proven to very useful during and after data collection

sessions. For example, during a reach and motion validation session using suited operations in the NBL (Neutral Buoyancy Lab), the prediction tool detected numerous typographical errors and measurement omissions. Motion analysis data along with collected multi-joint motion torque data is being used to validate the EMU kinematic and strength model. However, this work is not yet completed. The final report for this project will be completed in January 1998. The project funding terminated in September 1997.

The focus of this project is the understanding and modeling of the working envelopes, in terms of strength and motion, for EMU-suited humans. The goal is to achieve a practical, "lump parameter" approach to predict the maximum available strength for a given posture of a human working in an EMU suit in space. These specifics should guide our research through areas related to human performance in protective but constraining equipment such as diving suits, fire fighting suits, radiation protective suits, etc. In addition, because the approach taken with this research and development began in the physical therapy arena where there is interest in modeling maximal strength, posture, and motion to understand therapeutic strategies, the results of this activity will certainly be of interest to the physical therapy community. Finally, the statistical driven anthropometry prediction system developed under this task can be generalized to use other anthropometric databases for modeling humans both suited and unsuited for other human factors applications.

Human Task Performance Evaluation with Luminance Images

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-06-11-55

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1997

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$100,000

Responsible NASA Center: Johnson Space Center

Task Description:

This Space Human Factors proposal addresses a need to develop high-fidelity mockup and training simulators with the goal of providing cost-effective technology for facile evaluation of dynamically changing mission scenarios and training for operations in the harsh and rapidly changing lighting conditions of space.

NASA currently relies on ground tests to evaluate the influences of harsh on-orbit lighting conditions on Space Shuttle mission activities. These ground tests are expensive to carry out and must be repeated as mission parameters change in the course of planning. A more flexible and less expensive means to accomplish the same ends uses computer image computations of the lighting conditions in a space environment. The Graphics Analysis and Research Facility (GRAF) has demonstrated a predictive capability to construct maps of light intensity, called luminance maps, that agree with ground-test and on-orbit data. GRAF proposes to extend these luminance maps into simulated camera images that recreate solar-lit or artificially-lit on-orbit scenes as viewed by on-board cameras and evaluate whether these luminance images, when applied in a computer-based mission evaluation and training simulator, provide experience to the crew in handling the rapidly changing lighting conditions which will enhance their performance of tasks in space.

There are two stages of research necessary to provide this proposed capability, prior to application in a more complex training and mission evaluation environment. First, a software program to convert the luminance maps into simulated camera images to provide human interpretable TV pictures, needs to be developed and validated. Second, using a simple remote operator manipulation task, the degree of correlation of training with simulated camera images to task performance enhancement for mission operations needs to be determined. GRAF proposes to perform both stages of this research.

The first phase of the project has been completed. Two groups of ten subjects each were given training to align a target. The subjects in both groups tested with no time constraint and maximum accuracy performed the same with the "real" task. However, when subjects were constrained to align the targets in minimum time with maximum accuracy, a 35% percent increase in performance of the "real" task was measured for subjects trained with illumination as a factor. Subjective data was also collected. The results of this phase were presented at the 1997 Human-Computer Interaction International Conference. The second phase of the testing is in progress.

This phase will increase the degrees of freedom and the task complexity. This task is expected to be completed in April 1998 (due to late start with funding in 1996).

Because the modeling of illumination for use with training simulators may have an important effect on training in general, the benefits could be considered very general as well. For instance, tasks which may be performed during night hours such as operating a large ship, a truck, or an aircraft will be affected by visibility and less than perfect lighting conditions. Even a mundane operation such as driving an automobile will be affected. If training measures are identified which can make an operator more sensitive to these restrictions, the safer and smarter the operator will become.

FY97 Publications, Presentations, and Other Accomplishments:

Aldridge, A., Maida, J., and Novak, J. "Effects of lighting on human performance in training" in "Advances in Human Factors and Ergonomics." Edited by: Smith, M., Salvendy, G., and Koubek, R. Elsevier Press, volume 21B, pp 877-880 (1997).

Human Interaction Design for Cooperating Automation

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Funding:

UPN/Project Identification: 199-06-11-50

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$111,000

Responsible NASA Center: Johnson Space Center

Task Description:

The goal of this research is to improve human factors engineering for intelligent computer systems that support control center operators. The objectives are to develop and evaluate human-computer interaction designs, methods, and technology for networked workstations that support and automate real-time monitoring and anomaly detection, diagnosis, failure impact assessment, and malfunction procedure evaluation. These designs will support consistency and coordination between conventional telemetry monitoring software and automation software, including intelligent systems with advanced graphical interfaces. Designs will be developed for flight controller consoles in the NASA Johnson Space Center Mission Control Center. Another objective is to make advances in human factors methodology, to support the levels of analysis needed to design and evaluate intelligent automation systems to be "team players." Project products will include reusable designs, guidelines, and methods.

Human interaction designs, operational design prototypes, and system requirements were completed for two systems for aiding ground controllers in monitoring and reviewing events and situations. SPORT (Situation Playback Orbiter Data Reduction Complex (ODRC) Retrieval Tool) provides real-time data playback and plots for situation review, and automates data retrieval requests. The Situation Data Collection and Review System selects data for retrieval, presentation, and review in organized logs and plots. The situation review displays are designed to aid anomaly identification and comparison of expected with observed operations times and data during an event. The organized log design makes clear when expected data is missing and when a particular log entry is an inference made by the software, and uses an indented and expandable outline format so that the basis for an inference is accessible by expanding the detail below it. Thus, the user can easily distinguish inferences from data, and can see data underlying an inference without a separate capability for providing explanations. These designs have been adapted and implemented in the Space Shuttle Remote Manipulator System Assistant Project, as part of work to upgrade the space shuttle and to prototype advanced control center technologies.

One of the requirements that users levied late in the project is for a tool for developing specifications for the Situation Data Collection and Review System. The users did not focus on this requirement until the basic design was favorably evaluated. These users expect to develop and maintain the data files that underlie the creation of organized logs. Initial scenarios and storyboards have been developed for a specification tool for the

Situation Data Collection and Review System to incrementally develop specifications for capture and viewing of the situation data.

Documents that will be incorporated into the Field Guide for Designing Human Interaction with Intelligent Systems have been added to the Methods area of the CLARE library on the World Wide Web (<http://tommy.jsc.nasa.gov/~clare/methods/>). These include descriptions and examples of requirements, design, and evaluation products to document the methods of iterative analysis, design, and evaluation that are being used and refined by the team during the project. A multi-layered method of formal evaluation has evolved, which focuses on: (1) further understanding of the supported user tasks; (2) usefulness of strategies for supporting those tasks; and (3) usability of specific implementations of those strategies. A Guidelines and Lessons Learned document has also been drafted by collaborators from the Flight Crew Support Division to highlight the special features of the incremental methods used for designing usable and useful innovative aiding software, and to document new guidelines concerning capturing, logging, organizing, and analyzing system data.

Benefits to industrial and medical applications will be improvements in safety and effectiveness of automation software for operators of complex software-controlled equipment and processes. The innovative human-computer interaction design concepts, examples, and methods will advance human factors engineering knowledge and practice for complex multi-screen multi-application operations support systems.

FY97 Publications, Presentations, and Other Accomplishments:

Patterson, E.S. "Coordination across shift boundaries in space shuttle mission control." Technical report CSEL 97-TR-01, Cognitive Systems Engineering Laboratory, The Ohio State University (March, 1997).

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Roth, E.M., Malin, J.T., and Schreckenghost, D.L. "Paradigms for Intelligent Interface Design" in "Handbook of Human-Computer Interaction." Edited by: Helander, M., Landauer, T.K., and Prabhu, P. Elsevier North-Holland, 2nd edition (1997).

Watts, J.C., Woods, D.D., Corban, J.M., Patterson, E.S., Kerr, R.L., and Hicks, L.C. "Voice loops as cooperative aids in space shuttle mission control." Proceedings of the Computer Supported Cooperative Work '96, ACM, Boston, MA (November, 1996).

Perceptual Optimization of Image Compression and Displays

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Funding:

UPN/Project Identification: 199-06-12-39
Initial Funding Date: 1995
Students Funded Under Research: 4
FY 1997 Funding: \$279,000

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 2

Responsible NASA Center: Ames Research Center

Task Description:

NASA's ambitious plans for scientific observation of the heavens and Earth will generate vast quantities of image information, much of which will be compressed for storage or distribution to remote sites. Lossy compression techniques offer high compression ratios, but must be optimized for the relevant application. We have developed a novel and powerful technology for perceptual optimization of lossy compression. We propose a program of research to extend and enhance this technology (with university collaboration), and to apply it to several key applications in NASA and medical imaging (with NIH collaboration). In particular we will extend our technology to video compression (via the MPEG standard) and to wavelet compression. We will apply the technology to EOSDIS compression requirements, and to requirements of the National Library of Medicine. At the heart of our technology is a general model of human visual sensitivity. We also propose to continue enhancement of this model and to apply this model to the problem of optimizing the visual quality of displays.

Research Accomplishments

The first principal research contributions during the past year consisted of perceptual optimization of "wavelet" compression techniques. In a collaboration with UCLA, a model for visibility of quantization error and optimal compression techniques have been developed. This research has been the subject of three conference proceedings papers and one journal article.

The second principal research accomplishment during the past year was further development and validation of a new model of visual masking and contrast gain-control. This is the first explicit computable model capable of predicting masking in arbitrary digital images. An effort has also been made to apply the model to predicting target visibility in applied situations, such as runway obstacles. This research was the subject of three conference presentations, three proceedings papers, and one journal manuscript.

The third research accomplishment was a new theory of visual masking (entropy masking). This highly novel theory provides answers to some outstanding problems in understanding the visibility of targets on complex backgrounds. It has important applications in display design, visual communications, and predicting human visual performance in aerospace systems. It has resulted in two conference papers and six presentations.

A fourth research accomplishment was the continued development of new technology for perceptual optimization of Discrete Cosine Transform image compression algorithms. During the past year, Dr. Watson has extended this technology to the case of adaptive quantization, resulting in one proceedings paper. A second patent (5,629,780.) was received this year for this technology. This area has also received considerable technology transfer effort (see below).

A fifth accomplishment consists of successful application of temporal error diffusion algorithm to video sequence compression. This research answers an important technical question regarding bandwidth reduction for digital video. In the course of this research, we also successfully configured a real-time uncompressed video disk system. This allows leading edge research on digital video.

Technology Transfer

Dr. Watson has engaged in a number of collaborative technology transfer activities during the past year. With the ARC Commercial Technology Office and the Far West Regional Technology Transfer Center, he has prepared a Technology Opportunity Sheet for Watson's patented compression technology. He has also undertaken a collaboration with Tau Corporation to explore use of his patented technology for digital radiography. Most recently, he has initiated a collaborative project with the Desktop Video Group of Code II at ARC to apply his patented compression technology to NASA digital video applications. This has resulted in development of a portable demonstration software package that will be seeded to industry to encourage transfer. Finally, Dr. Watson has consulted with and advised twelve commercial entities on possible application of his patented compression technology.

Other PI Activities

During the past year, Dr. Watson was elected chair of the Vision Technical Group of the Optical Society of America, responsible for planning and execution of the Vision component of OSA Annual Meeting. He was also appointed to the organizing committee of the IEEE International Conference on Image Processing. He was asked to chair a Special Session of ICIP on "Image quality evaluation," and to chair a session on "Vision and Image Coding" of the SPIE/IS&T Human Vision and Electronic Imaging Conference. He also served on conference panels discussing "Perceptual Models" and "Quality and evaluation of imaging systems" (both at SPIE/IS&T).

During the past year the PI has also served on the Editorial boards of the journals *Journal of Mathematical Psychology*, *Displays: Technology and Applications*, and *Journal of Intelligent Systems*. Dr. Watson also authored or co-authored fifteen publications, including one US patent, six journal articles, and eight conference proceedings papers. He has given eleven scientific presentations, including five talks at major international conferences, three invited university lectures or colloquia, and three invited conference keynote speeches. The latter includes the keynote speech at the annual meeting of the Applied Vision Association in Dundee Scotland, and the opening talk of the Symposium on Imaging at Optical Society of America Annual Meeting, Rochester, NY. He has also provided high-level briefings to committees and visitors, including the NRC Commission on Engineering and Technical Systems, the Aeronautics and Space Engineering Board, the Advanced Technology for Human Support in Space, and the US Public Health Services, Office on Women's Health, on Advanced Technologies for detection, diagnosis, and treatment of breast cancer.

The Earth benefits of this research will be manifest in any enterprise that relies on visual communication of information. Significant examples are medical imaging, Earth resource imaging, space imaging, science imaging, and internet imaging. In each case, there is a need for efficient archiving and distribution of digital images, and high quality display of those images. Advances in medical imaging in particular may be expected to enhance diagnostic capabilities and to reduce costs of medical care. Earth resource imaging may be expected to reduce environmental damage and reduce costs of detection and repair of such damage.

In a more general sense, visual displays are at the heart of the modern technology revolution, from laptop computers and the world-wide-web to high-definition television, virtual reality, and telepresence. Improvements in the efficiency and quality of visual imaging and displays will have ramifications throughout our technological

infrastructure and economy. Beyond its technological payoff, the basic component of this research promises new understanding of the fundamental mechanisms of human vision, especially in the areas of visual detection and motion perception. This understanding will assist in analyzing visual diseases and injuries, and in developing appropriate therapies.

FY97 Publications, Presentations, and Other Accomplishments:

Ahumada, A.J., Jr. "Perceptual classification images from Vernier acuity masked by noise." (Abstract) *Perception*, 26(ECVP Supplement), 18 (1996).

Ahumada, A.J., Jr. "Simplified vision models for image quality assessment." *Society for Information Display International Symposium Digest of Technical Papers*, Morreale, J. (Ed.), pp. 397-400. Santa Ana, CA: Society for Information Display (1996).

Ahumada, A.J., Jr. and Beard, B.L. "Calibration of a spatiotemporal discrimination model from forward, simultaneous, and backward masking." (Abstract) *Perception*, 26 (ECVP Supplement), 34 (1997).

Ahumada, A.J., Jr. and Beard, B.L. "Image discrimination models predict detection in fixed but not random noise." *J. Optical Soc. Am., A* 14, 2470-2475 (1997).

Ahumada, A.J., Jr. and Beard, B.L. "Parafoveal target detectability predicted by local luminance and contrast gain control." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 8(4 (ARVO Suppl.)), S380 (1997).

Ahumada, A.J., Jr. and Beard, B.L. "Image discrimination models: Detection in fixed and random noise." *Proceedings, Human Vision, Visual Processing, and Digital Display VIII*, San Jose, CA, SPIE, Bellingham, WA, 3016 (1997).

Ahumada, A.J., Jr., Beard, B.L., and Watson, A.B. "Image discrimination models with contrast gain control predict target detection in fixed and random noise." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 37(3 (ARVO Suppl.)), S233 (1996).

Beard, B.L., Ahumada, A.J., Jr. Klein, S.A., and Slotnick, S.D. "Training on a vernier acuity task does transfer to untrained retinal locations." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 37(3 (ARVO Suppl.)), S696 (1996).

Beard, B.L. and Ahumada, A.J., Jr. "Orientation tuning unchanged by vernier acuity practice." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 38(4 (ARVO Suppl.)), S962 (1997).

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Beutter, B., Mulligan, J.B., and Stone, L.S. "The barberplaid illusion: Plaid motion is biased by elongated apertures." *Vision Res.*, 36(19), 3061-3075 (1996).

Eckstein, M.P., Ahumada, A.J., Jr., and Watson, A.B. "Visual signal detection in structured backgrounds. II. Effects of contrast gain control, background variations and white noise." *J. Optical Soc. Am.*, 14(9), 2406-2419 (1997).

Eckstein, M.P., Ahumada, A.J., Jr., and Watson, A.B. "Image discrimination models predict human detection in medical images." *Proceedings, Human Vision, Visual Processing, and Digital Display VIII*, San Jose, CA, SPIE, Bellingham, WA, 3016 (1997).

Eckstein, M.P., Ahumada, A.J., Jr., Watson, A.B., and Whiting, J.S. "What is degrading human visual detection performance in natural medical image backgrounds?" Proceedings, Medical Imaging, Newport Beach, CA, SPIE, Bellingham, WA, 3031 (1997).

Mulligan, J.B. "Application of M-JPEG hardware to dynamic stimulus production." *Spatial Vision* (In Press).

Mulligan, J.B. "Eye movements reveal hierarchical motion processing." *Invest. Ophthalmol. and Visual Sci.*, 38 (suppl.), S937 (1997).

Mulligan, J.B. "Eye-movement tracking using fundus images." (Abstract) *Optics & Photonics News*, 7(8 (Supplement)), 82 (1996).

Mulligan, J.B. "Image processing for improved eye tracking accuracy." *Behav. Res. Meth., Instr. & Comp.*, 29, 54-65 (1997).

Mulligan, J.B. "Application of temporal error diffusion to motion." *JPEG Proceedings, Human Vision and Electronic Imaging II*, San Jose, CA, SPIE, 3016 (In Press).

Rohaly, A.M., Ahumada, A.J., Jr., and Watson, A.B. "Object detection in natural backgrounds predicted by discrimination performance and models." *Vision Res.* (In Press).

Solomon, J.A. and Watson, A.B. "Cinematica: A system for calibrated, Macintosh-driven displays from within Mathematica." *Behav. Res. Meth., Instr., & Comp.*, 28(4), 607-610 (1996).

Watson, A.B. "Image quality and vision models." (Abstract) *Optics & Photonics News*, 7(8 (Supplement)), 134 (1996).

Watson, A.B. "The search for optimal visual stimuli." *Vision Res.* (In Press).

Watson, A.B. "Tutorial on image quality and vision models." (Abstract) *Optics & Photonics News*, 7(8 (Supplement)) (1996).

Watson, A.B. Compressing image data with minimal perceptual errors. *NASA Tech Brief*, 21(1), 73-74 (1997).

Patent Approved, U.S. Patent #: 5,629,780 Watson, A.B. "Image data compression having minimum perceptual error."

Watson, A.B. SPIE Invited speaker in panel on "Quality and evaluation of imaging systems" (February 11, 1997).

Watson, A.B. SPIE Invited speaker in panel on "Perceptual models: What's next." (February 10, 1997).

Watson, A.B. Applied Vision Association, Annual Meeting, Keynote talk on "Image Quality," Dundee, Scotland (1997).

Watson, A.B. Colloquium on "Visual Masking" at Cambridge University, England (1997).

Watson, A.B. Colloquium on "Image Quality" at Keele University, England (1997).

Watson, A.B. "Digital images and human vision." SPIE Invited speaker in session on "Perceptual models for image processing" (February 10, 1997).

Watson, A.B. "Perceptual image compression in telemedicine." Proceedings, Advanced Medical Image Compression, Storage and Transmission Technologies Workshop, Anaheim, CA, NASA (1996).

Watson, A.B. "Perceptual modeling of image quality." Proceedings, National Science Foundation Symposium on "High Fidelity Media Processing," USC National Science Foundation Engineering Research Center, Integrated Media Systems Center (1996).

Watson, A.B. and Rosenholtz, R. "A Rorschach test for visual classification strategies." Invest. Ophthalmol. & Visual Sci., 38(4 (ARVO Supplement)) (1997).

Watson, A.B. and Solomon, J.A. "A model of visual contrast gain control and pattern masking." J. Optical Soc. Am., 14, 2378-2390 (1997).

Watson, A.B. and Solomon, J.A. "Psychophysica: Mathematica notebooks for psychophysical experiments." Spatial Vision, 10(4), 447-466 (1997).

Watson, A.B., Borthwick, R., and Taylor, M. "DCTune perceptual optimization of compressed dental X-Rays." Proceedings, Medical Imaging, Newport Beach, CA, SPIE, 3031, pp. 358-371 (1997).

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Watson, A.B., Yang, G.Y., Solomon, J.A., and Villasenor, J. "Visibility of wavelet quantization noise." IEEE Transactions on Image Processing, 6(8), 1164-1175 (1997).

Human Interaction Design for Anomaly Response Support

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Funding:

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Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$110,000

Task Description:

The overall goal of this and a related project is to enable rapid improvement in human factors engineering for operations automation computer systems by developing immediately useful human-computer interaction (HCI) designs, methods, and technology.

The objectives of this research are, first, to develop and evaluate HCI designs for workstation-based systems that provide helpful information concerning anomaly detection and that support the fault management tasks of diagnosis, failure impact assessment, and malfunction procedure evaluation and monitoring. A second objective is to develop design concepts for a generic shell that will allow operations personnel to build anomaly response support systems for their particular application domains. A final objective is the development of methods for effectively designing and evaluating anomaly response support systems.

We have conducted a cognitive analysis of anomaly response which focused on the coordination occurring across a complex system of interdependent teams in space shuttle mission control. To date, we have conducted observations, interviews, and reviews of past anomaly cases. We observed the Mechanical, Maintenance, Arm, and Crew Systems (MMACS) flight control team during training simulations and missions, analyzed anomaly reports, as well as flight logs and mission books documenting past anomalies. We have also interviewed members of the MMACS team, as well as members of the MER (now called the MOIR) to further investigate the activities necessary for anomaly response. These activities have focused on the distributed nature of anomaly response in the space shuttle mission control domain, and have allowed us to develop a framework for our cognitive model, as well as ideas for aiding concepts to support anomaly response. These aiding concepts have been used to guide the design of a prototype distributed anomaly response support system.

To date, products include a general model of the cognitive processes involved in distributed anomaly response and a detailed description of anomaly response in the space shuttle mission control domain. We have completed two iterations of prototyping activity in order to explore initial aiding concepts for supporting anomaly response, as well as generic design concepts for supporting the development of anomaly response support across disciplines. These activities have led to the development of a web-based demonstration system called ARTIS (Anomaly Response Tracking and Integration System). A mockup based on these results can be viewed at <http://tommy.jsc.nasa.gov/~wong/hidars/hidars.html>. A more complete and robust prototype is currently being

implemented by our colleagues at NASA Johnson Space Center. We are currently in the planning stages of a user evaluation of this prototype system.

This research will benefit society in general by further developing our understanding of how to create tools to effectively support human problem solving. For example, by studying anomaly response in a distributed domain like space shuttle mission control, we are learning how to develop tools that effectively support group coordination and distributed problem solving. Our results should be applicable to many domains where problem solving is distributed across groups of people.

FY97 Publications, Presentations, and Other Accomplishments:

Watts, J.C. A cognitive analysis of functionally distributed anomaly response in space shuttle mission control. (Dissertation) The Ohio State University: Columbus, OH (1997).

Watts, J.C., Woods, D.D., Corban, J.M., Patterson, E.S., Kerr, R.L., and Hicks, L.C. "Voice loops as cooperative aids in space shuttle mission control." Proceedings of the ACM 1996 Conference on Computer Supported Cooperative Work (pp. 48-56). ACM Press: Boston, MA (1996).

Watts-Perotti, J.C. and Woods, D.D. "A cognitive analysis of functionally distributed anomaly response in space shuttle mission control." (Technical Report 1997-TR-02). Cognitive Systems Engineering Laboratory, The Ohio State University: Columbus, OH (1997).

Behavioral Trends and Adaptation During Space Analogue Missions

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Funding:

UPN/Project Identification: 199-16-11-14
Initial Funding Date: 1995
Students Funded Under Research: 0
FY 1997 Funding: \$125,000

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 0

Responsible NASA Center: Johnson Space Center

Task Description:

The proposed investigation is the first in a series of behavioral science studies designed to examine different aspects of psychological adaptation during long-duration missions, and in other isolated, confined environments. This investigation has two objectives: 1) identify and characterize trends in psychological and behavior variables over the course of long-duration analogue missions; and 2) obtain data to support the development of more specific hypotheses regarding psychological and behavioral changes in long-duration missions.

Many incidents reported during space missions flown by the United States and Russia have been attributed to reports of friction among crew members and lapses in judgment. A number of factors, such as isolation and confinement, are presumed to account for behavioral problems that occur in space. In order to understand and prevent undesirable changes, we must first ascertain the events and conditions that cause or influence these changes. Second, we need to measure the impact of behavioral and psychological changes in terms of health and performance readiness. Finally, we need to examine how individuals deal with behavioral and psychological changes when they occur. This descriptive study will use a pooled time-series approach to collecting and analyzing self-report measures of psychological and behavioral variables throughout.

By identifying and understanding aspects of psychological adaptation during long-duration missions and other isolated and confined environments, effective countermeasures and training can be developed that will also improve the safety, health, and well-being of non-space personnel on Earth. Personnel such as long-duration commercial divers, military personnel at remote outposts, or anyone living in isolated and confined environments for long periods of time will benefit from the information gleaned from this study.

Development of Data-Driven Models to Describe Astronaut Performance in Microgravity: Full-Body Dynamics and Control

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No Co-Is Assigned to this Task

Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 7

Post-Doctoral Associates: 0

FY 1997 Funding: \$101,683

Task Description:

The objectives of this research effort are to provide a quantitative approach to modeling microgravity system dynamics including the astronaut, Orbiter, support structure (i.e., RMS), and space hardware (i.e., spinning satellite of truss members). In addition to the appropriate zero-G dynamics, a detailed control model will provide the appropriate physiological performance of astronaut whole body motion. The Shuttle era has demonstrated numerous successful capabilities ranging from deployment of satellites, material science and life science experiments, planned and contingent extravehicular activity (EVA), and construction techniques. These successful operational systems capabilities are impressive, yet a void still remains. Experience has repeatedly shown that dynamic interactions between astronauts and systems they seek to manipulate can complicate the astronauts' task in unexpected ways. Rigorous analytical techniques need to be applied to solve dynamic interactions and control problems for astronauts' microgravity tasks. The resulting engineering mode will provide an assessment and simulation of human performance during space shuttle and station operations and will culminate in a modeling analysis package to assist in operations, planning, training, simulation, and advanced EVA techniques. This advanced analytical methodology complements the existing physical simulators (i.e., underwater training and the air bearing floor).

The current methodology includes computer programming, running experimental studies, and performing data analysis. The main software program is written to incorporate any specified object(s), solve the dynamic equations of motion, and then graphically display the results. The methodology was determined through demonstrations of Spartan mass handling and the Intelsat VI (mis)capture EVAs. These demonstrations display the utility of the analytical techniques. Experimental studies include motion analysis and muscle activation levels on a partial gravity simulator to assist in the development of an astronaut multi-segment model and space suit model. The research effort will yield an integration of astronaut dynamic motion and control strategies.

Newman, D.J. and Rahn, D.B.

A dynamic model of the extravehicular mobility unit (EMU) space suit was developed and applied to the simulation of several EVA tasks. The space suit model was created from mass, inertia, and torque data to augment the unsuited 12-segment human model used in previous studies. A modified Preisach model was

introduced to describe the hysteretic torque characteristics of joints in a pressurized space suit, and implemented numerically based on observed suit parameters. Four computational simulations were then performed to observe the effect of suit constraints on simulated astronaut performance, involving manipulation of the Spartan astrophysics payload on STS-63. It was found that the shoulder joint work required for a suited EVA crewmember to move the Spartan payload while in an inefficient posture was a full order of magnitude greater than the unsuited condition. Moving to a posture more accommodating to the suit's neutral position, the simulated astronaut completed the task with an actual decrease in shoulder work from the unsuited condition; to compensate for reduced upper-body mobility, however, the ankle joint was forced to use its long lever arm to manipulate the payload, resulting in ankle work 207% greater than in the unsuited condition. In this situation, muscle fatigue would set in more quickly than expected, a problem for the long-duration EVA missions expected on the International Space Station. The Spartan results agree with anecdotal evidence of post-EVA ankle fatigue, and demonstrate the effectiveness of both the space suit model and the simulation technique.

Newman, D.J. and Jackson, D.K.

Human mechanisms for control of posture and motion are normally optimized to perform in Earth's 1-G environment. The mechanisms by which the central nervous system controls and integrates posture and movement are the subject of considerable controversy and ongoing study. The control of jump landings is particularly interesting: (1) the control algorithm involves elements of preplanned trajectory formulation and sensory feedback; (2) dynamic interaction with the environment must be addressed; and (3) the controller exhibits rapid adaptation when exposed to changes in environmental or perturbation conditions. When modeling the human motor control mechanisms, these issues must all be accounted for at various levels of detail.

Exposure to microgravity in astronaut subjects provides a novel opportunity to test models of human motion control. Microgravity alters the body dynamics, placing considerably different demands on the controller. When astronauts adapt to space flight, they exhibit quantitatively different control strategies, often measured as postflight degradation in performance for balance, locomotion, and jumping. The research described here centers on the development of dynamic models of motor control for human jumping to provide a framework for testing three hypotheses:

1. *Postflight changes in astronaut jump landings result from alterations in commanded impedance.*
2. *The experimentally observed characteristics of downward jumps in humans can be captured by a control model based on an equilibrium point formulation.*
3. *An optimization procedure based on a set of physically and biomechanically plausible variables is sufficient to reconstruct key features of the experimentally observed trajectories for jump landings.*

Three experiments were designed to test these hypotheses, and were performed at Johnson Space Center, MIT, and the Massachusetts General Hospital. Several astronaut subjects were tested before and after space flight to determine the effects of microgravity adaptation on jump landing performance. A "moonwalker" experiment simulates short term partial gravity exposure to supplement the astronaut tests. A novel "false platform" protocol assesses virtual trajectory generation and impedance modulation during jump landings by measuring free limb trajectories when subjects unexpectedly fall through the landing surface.

Three models for the control of jump landings were developed: (1) a linear second order model of center of mass (CM) vertical motion; (2) a second order model of CM vertical motion using a knee joint; and (3) a planar three link inverted pendulum model. The false platform data are used in concert with these models to estimate body stiffness and damping properties immediately following the time of impact. Optimization techniques are used to interpret modeled jump landing trajectories.

Astronaut results indicate that reduced inflight postural control demands result in one of two responses postflight. Postflight-Compliant (5/9) subjects retain somewhat reduced leg stiffnesses after space flight, while Postflight-Stiff (2/9) subjects exhibit an overcompensatory increase in leg stiffness. Moonwalker subjects follow the Postflight-Compliant group, indicating that partial weight unloading provides a useful analogue for certain aspects of microgravity adaptation. Impedance estimates using the false platform data indicate negative

stiffnesses during the period immediately following impact. This implied instability suggests that such simple equilibrium point models cannot adequately describe jump landings. Optimization results capture the general features of the jump landing trajectories and indicate that controller requirements include impact force reduction as well as joint coordination to ensure appropriate mass center motion.

The computational multibody dynamics analysis package resulting from this research effort could be beneficial to the medical field if used to model altered balance responses to movement or jumping tasks (i.e., cerebral palsy, multiple sclerosis, and vestibular patients). The analysis package and computer simulations provide dynamic analysis as well as computer animation.

The EMU space suit model is easily extensible to confined-environment and suited activities on Earth, such as research and industrial diving, Antarctic investigation, and other extreme-environment tasks involving the constraints of an exposure suit.

At the basic biological level, this research effort has progressed in applying engineering adaptive control theory to model the central nervous system (CNS) and lower level involvement in the maintenance of posture. Again, these techniques provide a more rigorous analytical method for clinical use.

The analysis technique is also useful for 1-G, everyday concerns such as work injury analysis, clinical analysis, sports engineering, and real time computer analysis of most human motions.

FY97 Publications, Presentations, and Other Accomplishments:

Jackson, D.K. "Development of full-body models for human jump landing dynamics and control." (June, 1997).

Newman, D.J. "Investigating astronaut performance: Modeling and biomechanics." Orthopedics and Biomechanics Laboratory, Beth Israel Hospital, Harvard Medical School, Boston, MA (January, 1997).

Newman, D.J. and Barratt, M. "Life support and performance issues for extravehicular activity (EVA)" in "Fundamentals of Space Life Sciences." Edited by: Churchill, S. Krieger Publishing Co., Melbourne, FL, pp 337-364 (January, 1997).

Newman, D.J., and Jackson, D.K. "Altered astronaut performance following spaceflight: Control and modeling insights" in "Biomechanics and Neural Control of Movement." Edited by: Winters, J. and Crago, P. Springer-Verlag Publishing (In Press).

Newman, D.J., Jackson, D.K., and Bloomberg, J.J. "Altered astronaut lower-limb and mass center kinematics in downward jumping following space flight." *Exp. Brain Res.* (In Press).

Rahn, D. "A dynamic model of the extravehicular mobility unit: Human performance issues during EVA." (June, 1997).

Schaffner, G., Newman, D.J., and Robinson, S. "Inverse dynamic simulation and computer animation of EVA." AIAA-97-0232, AIAA 35th Aerospace Sciences Meeting, Reno, NV (January, 1997).

Distributed Decision Making in Extended Space Flight

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Post-Doctoral Associates: 1

Responsible NASA Center: Ames Research Center

Task Description:

The goal of this project is better understanding of team problem solving and decision making in order to enhance the ability of crews in space and on the ground to cope with unanticipated problems. Problems may relate to both physical systems and crew medical trauma. This need will be amplified on long-duration missions when no possibility exists for augmenting on-board resources or returning quickly to Earth, when communication with ground may be delayed or cut off, and with multicultural crews. The effects of several variables will be examined: team structure and expertise, cultural variability, and various stressors (isolation and confinement, ambiguity, and time pressure). We are most interested in how these variables affect problem diagnosis, risk assessment and management, conflict resolution, and decision making strategies. Products of this effort will include recommendations for procedures, systems, and training principles that enhance the quality of team decision making and performance.

1. Team Communication and Decision Processes. Previously, we developed a process model to characterize how teams make decisions in dynamic complex environments. The model has been modified to reflect the importance of time pressure and risk in the situation assessment component. These changes resulted from studies that showed (a) high salience of risk (both immediate and potential) to crew captains and of time pressure to all crew members, and (b) differences between crew positions in features of situations judged to be most important to decision making. Accurate risk assessment is critical to good decision making, especially when the situation is ambiguous and is changing dynamically. Apparent underestimation of risk has been associated with many accidents involving human judgment. This year's work has focused on alternative perspectives on risk and the role of risk perception on decision making. Risk perception has been shown to be a function of both the degree to which the consequences of decisions in risky situations affect oneself (as opposed to others) and one's perception of control over the situation. A study has been designed to assess differences between pilots and controllers in risk perception and recommended actions in flight situations. Locus of control and focus of consequences will be varied. Materials have been developed and data collection is about to commence. Data

have been collected from ground-based dispatchers on the types of risk they perceive in their jobs. These data will be used for constructing decision scenarios to be used in future studies.

2. **Cultural Diversity and Crew Communication.** Future space missions will include more diverse crews than present missions, both in terms of native cultures and gender. Despite high levels of training, deeply ingrained norms for interacting with peers, superiors, and subordinates may lead to conflicts and misunderstandings. This is likely to be most problematic when emergencies occur or problems arise from errors or oversights on the part of a crew member. For long-duration space missions, it will be essential to identify effective and trainable communication strategies. Studies are underway to understand cultural differences in the language used by crew members to call attention to a problem or to an error committed by the other crew member, who may be of a higher or lower status. Flight-related scenarios have been developed that differ in level of risk and in the degree to which “face” is challenged. Two baseline studies have been completed in the US. These involved pencil-and-paper responses to the problem scenarios. Results from the first study showed that US captains were more direct in addressing first officers than were first officers in addressing captains. Moreover, communication was less mitigated (more direct) in high-risk situations than in low-risk situations for both crew positions. Study Two compared female air carrier pilots with male pilots matched in terms of years and type of aircraft experience. Study Two found that crew position is more important than gender in the type of communication strategies used. All captains, regardless of gender, used more direct communication than did first officers. However, female captains backed up their commands with supporting statements or justifications more often than did male captains, who tended to rely on their status. Data collection has been completed with an English-speaking foreign carrier, as well as with two non-English speaking foreign carriers. These data are now being analyzed. A third US baseline study involved actual verbal responses to the scenarios described in the written protocols. The events were embedded in flight scenarios presented to professional pilots “flying” the NASA 747-400 flight simulator. Videotapes of crew interaction are just being analyzed. The next phase of the study will assess the effectiveness of various communication strategies, both in the US and in countries that differ from the U.S. in power distance and group orientation.

3. **Remote Diagnosis for Trauma Patient Resuscitation.** This study addresses the cognitive demands of distributed medical decision making as it pertains to the treatment of acute trauma patients. Telecommunication systems have enabled experts to offer consultation to spatially distant locations. A series of experiments was conducted to investigate (a) how experts with different experience backgrounds (nurses, surgeons, and anesthesiologists) extract information from audio-video sources and (b) how they deal with the uncertainties presented by acute trauma cases. In the first study, decision makers were presented with audio-video case segments of trauma patient resuscitation. They were asked to describe current and anticipated patient status and team activities. Overall, anesthesiologists were more accurate and their responses were more elaborated than those of the other two groups. This finding may reflect the emphasis in the selected cases on the role the anesthesiologists play in immediate patient stabilization. Nurses consistently focused more on teamwork than the other groups. These findings suggest that experts with different experience backgrounds may appreciate different aspects of the events and activities presented in audio-video sources. In the second study, over 40 video case segments were analyzed by care providers to answer the question “What is uncertain to the team related to this case segment?” Responses fell into two major categories. The first pertained to uncertainty about the site and extent of patient injury. Such uncertainty was complicated by uncertainty about the patient’s prior medical history, working status of patient monitors, and uncertainty about the effect of treatment. The second class of uncertainty pertained to team and organizational factors. These uncertainties involved task distribution among team members, availability of team members, intentions of other team members, and treatments already applied. In general, the patient-related uncertainties were more tractable to the professionals. The team-related uncertainties stemmed from poor communication among the team members about intentions, plans, and actions. Non-standard organizational factors contributed to these uncertainties. These findings have implications for establishment of procedures and training to facilitate effective remote team diagnosis.

4. **Effects of Prolonged Isolation on Team Decision Making.** Little systematic evidence exists on how prolonged isolation affects team performance on types of tasks that will be especially critical for space missions--challenging, complex tasks that require team coordination for success. This study will examine the

effects of isolation and confinement on team decision making in Antarctic overwintering personnel. Behaviors that may be sensitive to isolation effects include risk taking, cooperation and competition, resource and information sharing, dynamic workload distribution, and team coordination. A Distributed Dynamic Decisionmaking tool (DDD-III) has been adapted for use in Antarctica. A search and rescue task has been developed with modifications in terrain, risk, and uncertainty levels in order that the task may be played repeatedly over the course of the winter. Final instructional packages are being tested.

Earth benefits from this project are expected in three areas: training, design of procedures to enhance team decision making, and specification of requirements for decision aids. Training will apply both to individuals and teams that operate in dynamic high-risk environments. The decision process model developed under this grant is most directly applicable in the aviation domain, where pilots, air traffic controllers, dispatchers, and maintenance specialists often must pool their resources to solve problems. To date, several airlines have adopted our dynamic decision process model as a framework for training their flight crews to assure greater safety within the aviation system. Findings are also being applied in other industries where technical specialists and managers must cope with problems and make decisions, such as management of off-shore oil platforms, nuclear power operations, and fire fighting.

The analysis of cultural and gender factors in effective team communication will be relevant for designing procedures to facilitate and support communication in multicultural settings in many technical domains, including air traffic management. Likewise, results of the effects of stress identified in overwintering Antarctic teams' performance may provide insights to support other Earth-based teams operating in high-stress environments.

Findings from our remote medical diagnosis effort will be directly applicable to telemedicine on Earth, where medical practitioners cannot observe patients first hand due to their remote location or where a distant specialist may be required. This project will yield information about what kind of information, structure of inquiry, and information support tools are most useful for remote diagnosis, tailored to the level of knowledge and expertise of the practitioner.

FY97 Publications, Presentations, and Other Accomplishments:

Fischer, U. and Orasanu, J. "How to challenge the captain's actions." Proceedings of the Ninth International Symposium on Aviation Psychology, Jensen, R. (ed.). Mahwah, NJ: Lawrence Erlbaum Associates (1997).

Gibson, J., Orasanu, J., Villeda, E., and Nygren, T.E. "Loss of situation awareness: Causes and consequences." Proceedings of the Ninth International Symposium on Aviation Psychology. Jensen, R., (ed.). Mahwah, NJ: Lawrence Erlbaum Associates (1997).

Orasanu, J. "Stress and naturalistic decision making: Strengthening the weak links" in "Decision Making Under Stress: Emerging Themes and Applications." Edited by: Flin, R., Salas, E., Strub, M., and Martin, L. Avebury (In Press).

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Orasanu, J., Fischer, U., and Davison, J. "Cross-cultural barriers to effective team communication" in "Cross-Cultural Work Groups." Edited by: Oskamp, S. and Granrose, C. NY: Sage (1997).

Orasanu, J., McCoy, E.C., Davison, J., Smith, P., Owsley, T., Rodvold, M., Bullington, R., and France, L. "Managing irregular operations in a free flight environment." Proceedings of the Ninth International Symposium on Aviation Psychology, Jensen, R. (ed). Mahwah, NJ: Lawrence Erlbaum Associates (1997).

Xiao, Y. and Mackenzie, C.F. "Uncertainty in trauma patient resuscitation." Proceedings of the Human Factors and Ergonomics Society 41st Meeting. Santa Monica, CA: HFES (1997).

Xiao, Y., Mackenzie, C.F., and Spencer, R.L. "The effect of experience background on remote diagnosis." Proceedings of the Human Factors and Ergonomics Society 41st Meeting. Santa Monica, CA: HFES (1997).

Antarctic Space Analog Program

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Funding:

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Students Funded Under Research: 0
FY 1997 Funding: \$43,905

Solicitation: 96-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 0

Task Description:

The primary aim of the proposed project is to examine group dynamics and individual performance in extreme, isolated environments and identify human factors requirements for long-duration space missions using data collected in an analog environment. Specifically, we wish to determine: 1) the characteristics of social relations in small groups of individuals living and working together in extreme, isolated environments, and 2) the environmental, social and psychological determinants of performance effectiveness in such groups. These two issues will be examined in six interrelated studies using data collected in small, isolated research stations in Antarctica from 1963 to the present. Peer nominations of fellow crewmembers on a number of different dimensions will be used to in cross-sectional and longitudinal assessments of group dynamics defined in terms of group structure and centrality. The proposed project responds to two NASA program emphases for FY 1997 as described in the NRA: 1) the primary emphasis of the Behavior and Performance Program on determining long-term individual and group performance responses to space, identifying critical factors affecting those responses and understanding underlying mechanisms involved in behavior and performance, and developing and using ground-based models and analogs for studying space-related behavior and performance; and 2) the emphasis of the Data Analysis Program on extended data analysis. Results from the study will be used to develop recommendations for the design and development of pre-flight crew training and in-flight psychological countermeasures for long-duration manned space missions.

In the last quarter of FY97, two major data sets were created for analysis: the Operation Deep Freeze file consisting of data collected on 657 American men who wintered over as members of 42 different expeditions at six small Antarctic research stations between 1963 and 1974, and the Antarctic Debrief file consisting of data collected on 300 American men and women who wintered over at McMurdo and South Pole Stations from 1991 to 1997. A study of the influence of crew size on the relationship between social dynamics and individual performance found that smaller winter-over crews appear to be more useful analogs than larger crews of social dynamics and individual performance during long-duration space missions.

This research seeks to understand fundamental processes of social interaction and social dynamics in small groups in isolated and confined environments (ICEs). These include both long-duration missions in space and

such ground-based environments as polar research stations, nuclear submarines, isolated military outposts and mining communities, and offshore oil rigs. The results of this research will assist in the development of “select-in” methods for screening and selection of personnel to live and work in space and other isolated and confined environments for prolonged periods. The results will also assist in the development of training programs and psychosocial countermeasures designed to enhance group performance and cooperation and to minimize the risk of interpersonal conflict during long-duration missions in space and other isolated and confined environments. Research results will also help to elucidate the relationship between patterns of social organization and interaction and individual behavior and performance in small groups.

*Review and Analysis of Diaries from French Remote Duty Stations***Principal Investigator:**

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No Co-Is Assigned to this Task

Funding:

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Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

This research project involves the translation and analysis of personal journals that were maintained for this purpose by the station leaders and medical officers of the Dumont d'Urville Antarctic facility and other French remote duty stations located on small islands in the South Indian Ocean. NASA initiated this collaboration with CNES and the Territoire des Terres Australes et Antarctiques Françaises (TAAF) in 1993. The diaries were maintained by remote duty personnel during the 1993-1994 mission as part of the International Antarctic Psychological Program (IAPP). This project builds upon previous research by the principal investigator concerning the behavioral issues associated with isolation and confinement. The objective of the study is to develop further understanding of the human requirements for long-duration space exploration.

The principal investigator and Dr. Claude Bachelard (medical director of TAAF) together translated and reviewed the diaries, and performed the Level I allocation of entries to the list of behavioral issues. Three additional categories emerged during this task. The modified inventory of issues is presented below.

- | | |
|---------------------|-----------------------------------|
| • Sleep | • Organization |
| • Safety | • Habitat aesthetics |
| • Exercise | • Recreational/leisure |
| • Clothing | • Personnel selection |
| • Workload | • Scheduling/workload |
| • Leadership | • Privacy/personal space |
| • Medical support | • Outside communications |
| • Personal hygiene | • Internal communications |
| • Food preparation | • Permission training/preparation |
| • Group interaction | • Waste Disposal and Management |

The principal investigator coded and entered the data into computerized spreadsheets. The spreadsheet configuration was designed to permit each relevant diary entry to include the diarist's name, role (i.e., leader or medical officer), the mission day, a code for the primary category of behavioral issues, a code for a secondary category (if applicable), the page number of the diary, and the translated text of the entry.

Linking mission day, issue category, and role codes to the entries will permit sorts to be made that arrange diary entries in terms of the key variables. The results of the data sorts will serve as the basis for the Level II analysis. Specifically, all diary entries will be combined into a single database, then sorted by primary category, then by secondary category, and finally by mission day. The resulting arrangement of the database will permit the identification and quantification of issues within the categories; issue salience and any correlations between specific issues and mission phase also will be identified.

The results of this research primarily will be useful to the planners and managers of remote duty stations, on Earth and in space, and to the crew personnel who live and work in isolation and confinement. The potential benefits, however, are not limited to special duty conditions. In a very real sense we are all crew members onboard a space ship, and we might all learn how to better adapt to our conditions, and get along with each other, by studying examples of groups that have succeeded and failed under circumstances far more difficult than our own. In important ways, studying small groups in isolation and confinement is like viewing society through a microscope. There is much of general value to learn from this approach.

FY97 Publications, Presentations, and Other Accomplishments:

Stuster, J. "Bold endeavors: Lessons from polar to space exploration." Invited presentation to the Committee on Space Biology and Medicine of the National Academy of Sciences (August, 1997).

Stuster, J. "Human adjustment to isolation and confinement." Proceedings of the 27th International Conference on Environmental Systems, Lake Tahoe, NV (July, 1997).

Stuster, J. "Human and team performance in extreme environments: Antarctica." Proceedings of the International Symposium on Aviation Psychology, Columbus, OH (June, 1997).

Spatial Auditory Displays for Space Missions

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Post-Doctoral Associates: 0

Responsible NASA Center: Ames Research Center

Task Description:

An integrated basic research, applied research, and technology development program is proposed with the goal of successfully implementing three-dimensional (3-D) auditory displays for improved operator efficiency and safety. The program is best described as a double effort: 1) to conduct perceptual studies of human sound localization using techniques developed for real-time synthesis of 3-D sound over headphones using measurements of Head-Related Transfer Functions (HRTFs) from individual subjects; and 2) to use the critical knowledge gained in the course of the basic research that is required for both enhancing and perceptually validating the advanced acoustic display systems that have been developed as part of the ongoing spatial sound project at NASA Ames Research Center (ARC). The two-ear (binaural) listening system enables an astronaut, ground-controller, or other human operator to take advantage of their natural ability to localize sounds in 3-D space. Synthetic localization of acoustic objects in information displays can be used to enhance situational awareness, to improve segregation of multiple audio signals through selective attention, and to provide a means of detecting a desired signal against noise for enhanced speech intelligibility. Auditory cues can provide a critical channel of information when visual cues are degraded or absent in space operations such as telerobotic assembly and repair, proximity operations, management of complex on-board space station systems, speech communications, and enhanced virtual environment displays for ground-based training.

Deliverables for this project include human factors guidelines for the development of virtual acoustic displays, in the form of refereed publications, conference papers, and technical reports. Deliverables for the advanced technology development effort may also include algorithms and hardware/software implementations for measuring HRTFs in arbitrary environments and rendering efficient algorithms for high-performance spatial sound synthesis including real-time, complex room modeling. Software is also being developed which enables experimental control of spatial sound parameters for psychoacoustical experiments such as the number and placement of reflections and their level of fidelity.

Progress to date for the third grant-year includes completion of four psychophysical studies. Seven articles or book chapters, six conference papers, and sixteen technical presentations also resulted. These experiments investigated localization performance for virtual sources both with and without head and/or source motion and as a function of various manipulations of the interaural time and intensity cues in the stimuli. In addition, one study was published which determined echo thresholds using non-headtracked stimuli in which the number and salience of dense late reflections was manipulated. In addition, the threshold was evaluated for late reflections themselves. Another study is also underway which investigates the threshold of reverberation in reflective environments as a function of room reverberation time and the amount of energy in different octave bands. This study required additional development of software-hardware systems for prediction, evaluation, and accurate simulation of the character of an acoustic environment.

Work also began which explores the impact that various virtual environment system parameters, such as system latency and update rate, may have on listeners' perception. For example, examination of the head motions that listeners used to aid localization in the study described above suggests that some head motions may be as fast as about 400°/sec for short periods of time. Analysis of latencies that are present in typical virtual systems suggests that: (1) commonly specified parameters such as the audio update rate determine only the "best-case" latency possible in a virtual audio system (VAE); (2) total system latency and individual component latencies are frequently not measured by developers of VAEs; and (3) typical system latencies may result in under-sampling of relative listener-source motion of 400°/sec as well as positional "jitter" in the simulated source.

Continued work in the area of technology development included: refinement of the Crystal River Engineering "Snapshot" HRTF measurement system at NASA Ames and initial development of an *in situ* surface transfer function measurement system for diffuse field modeling. A modification of the Snapshot system to integrate head-position sensor information into the measurement chain is complete. Progress has also been made on developing software extensions to allow measurement of materials properties. We also began the development of basic software capabilities to enable the synthesis of reflection cues in dynamic contexts. Planning and initial software development for this project has been accomplished. The hardware platform chosen shifts the primary implementation role to software and uses general purpose CPUs (dual Pentium II with MMX capability) as the hardware platform. The signal processing algorithms are first being implemented in Matlab and then ported to C++ for conducting experiments. Progress was also made on developing techniques for measuring and rendering (nonreal-time) room responses for larger environments. This capability will enable us to more effectively study the detectability of reflections in reverberant environments as well as the role reverberation plays in localization accuracy and speech intelligibility.

The work overall enables the eventual implementation of a fully-functional, real-time implementation of a virtual acoustic environment. We have made significant advances in the calibration of measured acoustical spaces to equivalent, synthesized virtual acoustic spaces. Among the major findings so far are: (1) the added computational expense of real-time implementation of head tracking for source and/or head movement is worthwhile if minimization of image 'reversals' is necessary—a reduction in reversal rate from 23% to 6% was found for normal HRTFs (all interaural cues present) and 34% to 28% for interaural time cues alone; and (2) typical virtual system latencies may result in under-sampling of observed listener-source motions of about 400°/sec as well as positional "jitter" in the simulated source.

The 3-D audio research activities conducted at ARC under this grant have brought together a new understanding of the basic perceptual mechanisms of auditory localization, and the incorporation of this understanding into technologies for improving the safety and quality of audio communication. This is accomplished by digitally capturing, and then modeling, the acoustic features of both humans and their acoustic environment. Such modeling advances the development of improved human interfaces that address communication transfer problems in both space and Earth contexts.

We have developed several base technologies for enabling virtual acoustic displays applicable to both space operations and to the commercial sector. An example is the Crystal River Snapshot system for measuring HRTFs of individuals in reflective environments. Previous to the development of the Snapshot system, the

measurement of HRTFs was very costly in terms of time and equipment, and required the use of specialized facilities such as an anechoic chamber. The Snapshot system enables measurements in normal reflective environments such as an office and utilizes a standard PC and sound card. Another example is the US patent awarded in 1995 for "Multi-Channel Spatialization System for Audio Signals." This device enables communication personnel to use their inherent ability to segregate, monitor, and switch attention among multiple communication channels (as many as seven radio communication channels are monitored simultaneously during NASA shuttle launch operations). We fabricated virtual acoustic display prototypes based on this patent for both Kennedy and Johnson Space Centers. Desired signal levels can be heard at a lower volume against background noise and intelligibility is improved, contributing towards less fatiguing and safer operations. Recently, several NASA technology transfer centers have been working to license this technology for hardware used in similar high-stress applications, including 911 operator consoles and aviation communications. The technology is also extendible to teleconferencing, a major commercial application area of 3-D sound technology. Yet another example is the room modeling research we have conducted. The goal is to be able to predict the acoustics and noise levels within a structure before it is built using both prediction software for room modeling and auralization hardware. Such a system also enables virtual listening within the modeled room, and comparison with changes in wall materials, number of noise sources, etc. Once a particular room has been modeled, we can conduct psychoacoustic experiments to determine how to best modify an acoustical situation for a purpose such as noise reduction. Psychoacoustic methods are used to measure speech intelligibility or other parameters, potentially within a modeled space shuttle laboratory or a modeled conference room on Earth. Finally, the basic research we have conducted in head movement and localization allows our auditory displays to include all of the relevant perceptual and acoustic mechanisms that constitute auditory localization, thereby improving human performance within interactive systems. This work provides developers with the means to improve auditory displays for many different applications, especially those within virtual reality. These include teleoperation, telecommunication, human-machine interfaces, simulation, communication, and design and medical facilities.

FY97 Publications, Presentations, and Other Accomplishments:

Begault, D.R. "Fundamentals of acoustics. Psychoacoustics and hearing. Acoustical measurements" in "Acoustics and the Built Environment." Edited by: Salter, C.M. New York: McGraw-Hill (In Press).

Begault, D.R. "Virtual acoustics, aeronautics and communications." (invited paper) 101st Convention of the Audio Engineering Society, Los Angeles, CA. New York: Audio Engineering Society, Preprint 4352 (November 8 - 11, 1996).

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Begault, D.R. "Virtual acoustics: evaluation of psychoacoustic parameters of early reflection synthesis for improved rendering and data reduction." [Invited paper] Proceedings of IWHIT'97, Third International Workshop on Human Interface Technology, University of Aizu, Aizu-Wakamatsu, Japan (March 12 - 14, 1997).

Ellis, S.R., Begault, D.R., and Wenzel, E.M. "Virtual environments as human-computer interfaces" in "Handbook of Human-Computer Interaction." Edited by: Helander, M. Amsterdam: Elsevier Science Publishers (1997).

Macpherson, E.A. "A comparison of spectral correlation and local feature-matching models of pinna cue processing." J. Acoust. Soc. Am., 101, 3104 (1997).

Macpherson, E.A. "Effects of source spectrum irregularity and uncertainty on sound localization." (Abstract) J. Acoust. Soc. Am., 99, 2515 (1996).

Shinn-Cunningham, B.G., Lehnert, H., Kramer, G., Wenzel, E.M., and Durlach, N.I. "Auditory displays" in "Binaural and Spatial Hearing in Real and Virtual Environments." Edited by: Gilkey, R. and Anderson, T. Mahwah, NJ: Lawrence Erlbaum, pp 1-25 (1997).

Wenzel, E.M. "Effectiveness of interaural delays alone as cues during dynamic sound localization." (Abstract) *Acoustical Soc. Am.*, 100, 2608 (1996).

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Wenzel, E.M. "Analysis of the role of update rate and system latency in interactive virtual acoustic environments." 103rd Convention of the Audio Engineering Society, New York, NY, preprint 4633 (September 26 - 29, 1997).

Wenzel, E.M. "What perception implies about implementation of interactive virtual acoustic environments." 101st Convention of the Audio Engineering Society, Los Angeles, CA. Preprint number 4353 (November 8 - 11, 1996).

Wenzel, E.M. "What perception implies about implementation of interactive virtual acoustic environments." Proceedings of IWHIT'97, Third International Workshop on Human Interface Technology, University of Aizu, Aizu-Wakamatsu, Japan (March 12 - 14, 1997).

Wightman, F.L. "Sound localization in the presence of multiple distracters." *J. Acoust. Soc. Am.*, 101, 3105 (1997).

Wightman, F.L. and Kistler, D.J. "Factors affecting the relative salience of sound localization cues" in "Binaural and Spatial Hearing in Real and Virtual Environments." Edited by: Gilkey, R. and Anderson, T. Mahwah, NJ: Lawrence Erlbaum (1997).

Wightman, F.L. and Kistler, D.J. "Individual differences in human sound localization behavior." (Abstract) *J. Acoust. Soc. Am.*, 99, 2470.

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Physiological Effects of Decompression-Induced Venous Bubbles

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Funding:

UPN/Project Identification: 199-04-17-11 Solicitation: 93-OLMSA-07
Initial Funding Date: 1995 Expiration: 1998
Students Funded Under Research: 1 Post-Doctoral Associates: 1
FY 1997 Funding: \$100,859

Task Description:

Venous air bubbles result from moderate (less than 20,000 ft) decompression to altitude. Known consequences are: vascular obstruction, vasoconstriction, diffuse pain especially around joints, inflammation, edema, and recurring injury to the vascular endothelium. Neurological symptoms can result if venous bubbles become arterIALIZED and embolize the central nervous system. Less known consequences involve the release of vasoactive and permeability altering biochemical mediators, especially from the lungs which are the principal target organ for the venous bubbles, and from activated cells, including neutrophils. These mediators include the prostaglandins, thromboxanes, and leukotrienes. Astronauts involved with extravehicular activities (EVA) are at risk for decompression sickness. Risk is estimated as high as 20% based on extensive ground-based studies. Although the operational incidence of qualitative symptoms of decompression sickness in space is remarkably low, this is due in large part to the extensive preventative measures that are undertaken just prior to an EVA. Such measures include a staged decompression as well as oxygen prebreathing to washout tissue nitrogen stores. With development of the space station, EVA prevention measures may change, as well as the risk of repetitive exposures. The evaluation of quantitative biochemical markers may be more sensitive than decompression sickness symptoms and afford new opportunity to better assess risk of physiological decompression stress. The work proposed addresses the investigation and evaluation of quantitative indices of decompression-induced physiological stress using proven experimental models. The results of these studies will enable better assessment of the physiological risk of decompression sickness and begin to establish utility of operational monitors using body fluids such as blood or urine for quantitative evaluation.

The efforts accomplished thus far include identification and incorporation of specific and appropriate enzyme immunoassay techniques to quantitate the change in bioactive mediator (eicosanoid) levels with decompression and venous air embolism. Specific to FY 97 have been A) the inhibition studies using dibutyl C-AMP to determine if blocking both pathways leading to production of leukotrienes and thromboxane is effective in attenuating the inflammatory response to decompression stress, and B) identification and validation of urine as an appropriate sample fluid that may afford use as an operational or clinical marker.

The questions answered thus far include the identification and utilization of appropriate analytical methodologies to define some of the bioactive mediators involved with decompression stress and to initiate studies aimed at specifying what pathways of mediator production are involved.

New questions have arisen that relate to the possibility of attenuating the activation of specific cell types that are involved in the release of the inflammatory mediators as a result of decompression stress, and further, as to whether endogenous chemicals modulate these responses.

The future work on this task can take us into the area of intervention of inflammatory response to decompression bubbles and to examine the role of other factors such as diurnal hormone levels, etc. on these responses.

The disease malady that this research is based upon has an Earth counterpart, specifically decompression sickness that occurs in sports divers, commercial undersea divers, and aviators (civilian and military) flying at high altitudes. The particular insult being studied involves the effect of venous air embolism on the organism which causes circulatory changes and organ dysfunction. There is a close clinical counterpart to this particular illness, namely clinical air embolism that is commonly reported with open-heart surgery, neurosurgery, and in specific intensive care unit patients who require mechanical ventilation.

A clearer understanding of the hemodynamic and biochemical changes (including hematological evaluation) of venous air embolism can certainly benefit the prescribed efforts that are useful in evaluating and treating the clinical disease. The endpoint to effective therapy includes not only the evaluation of the insult (diagnostic) but also the delineation of the specific damaging agent. In the present effort, the identification of the particular eicosanoids involved in the expression of decompression illness and the evaluation of the injury will help specify any adjunctive action that may complement routine protocols. For example, use of a drug that specifically blocks or inhibits release of damaging agents, or one that prevents the activation of certain cell types, might aid in the treatment process or at least hasten recovery.

The impact of these results can provide clearer understanding of the mechanism of decompression illness and clinical venous air embolism. The degree of organ injury and the particular bioactive mediator involved will offer new opportunities to effect appropriate and specific therapy.

FY97 Publications, Presentations, and Other Accomplishments:

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Carbon Dioxide-Oxygen Interactions in Extension of Tolerance to Acute Hypoxia

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Funding:

UPN/Project Identification: 199-14-17-14
Initial Funding Date: 1994
Students Funded Under Research: 2
FY 1997 Funding: \$120,000

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 0

Task Description:

Studies in this and other laboratories have shown clear improvement in useful consciousness of normal men at rest when atmospheric CO₂ partial pressure is increased during the impaired consciousness caused by atmospheric hypoxia. The overall project objective is to obtain on-line dynamic quantitative physiologic measurements, of respiratory, gas transport, and brain circulatory factors, that contribute to acute improvement in mental function during rest and physical work in hypoxic environments. A specific further purpose is to provide this information for predictive modeling of rates and degrees of acute adaptation and deadadaptation to hypoxia, producible by control of inspired CO₂. NASA relevance is to accidental or intentional exposure to hypoxic atmospheres in any aspect of present or long-range manned space activity, including space station, spacecraft, or Earth-bound compartments or structures.

This program had its origin in a previous recognition that in brief stable state exposures to reduced inspired PO₂, the addition of carbon dioxide to the inspired gas improved arterial and brain oxygenation, and sustained aspects of mental and sensory performance.

The present project has the objective of measuring rates and degrees of human physiologic adaptation to acute inspiratory hypoxia, at rest and in physical work. An initial series of experiments at one ATA compared the effects of 12 and 10% O₂ on several tests of cognitive and psychomotor function, to select tests highly sensitive to hypoxia. Test of Choice Reaction Time and Numeric Reasoning functions were found to be diminished by 10% but not by 12% O₂ exposure. This allowed experimental measurement of the effect on the selected neural functions of carbon dioxide (4%) added to 10% inspired oxygen. The addition of carbon dioxide prevented the hypoxic depression of mental and psychomotor test scores, in rest, and in 50 Watt exercise.

During the Project immediate prior year the systematized capability was improved for simultaneous breath-by-breath measurement of pulmonary ventilation and end-tidal alveolar gas composition, and heart beat-by-heart beat monitoring of arterial blood hemoglobin saturation (pulse oximeter), cardiac frequency, and

middle cerebral artery blood flow velocity (transcranial doppler). These methods were employed in a series of experiments to compare the dynamic physiologic responses to abrupt exposures to 10% and to 12% inspired O₂, without and with 4% CO₂ to prevent hypoxic hypocapnia. The project allowed examination of the rapid initial rates of change of respiratory parameters and the brain blood flow index (MCA Flow Velocity) as these related to dynamic changes in arterial oxygenation and end-tidal "alveolar" carbon dioxide pressure. These experiments demonstrated that arterial blood oxygenation in exposure to 10% O₂ with 4% CO₂ is superior in degree and stability to breathing 12% O₂ alone, without causing end-tidal carbon dioxide partial pressure to exceed the normal resting level.

The current year has included extraction and organization of breath-by-breath and cardiac beat-by-beat data, from each of the cited experiment series to date for the mathematical and statistical evaluation of data averaged at intervals of one minute or less for dynamic analysis. The critical importance of carbon dioxide and oxygen interacting influences on both respiration and brain blood flow in acute hypoxia made it necessary to effect improvements in pulse oximeter calibration for determination of % Hb Saturation in low degrees of arterial oxygenation. This is being accomplished both in collaboration with the pulse oximeter manufacturer, and by specific experiment in subjects breathing nitrogen mixtures with low oxygen pressures (12 and 10% O₂, in rest and exercise).

This research concerns the fundamental intrinsic physiological adaptations to sudden decrease of oxygen in the inspired air. The situation occurs in fact or potentially in industrial, aerospace, undersea, military, medical, and special natural environments. The research includes determining methods for using harmless levels of carbon dioxide to accelerate and improve the degree of tolerance to hypoxic exposure. A goal is to determine the basic dynamic interrelationships of the multiple physiologic control systems which influence respiration and blood, brain, and heart oxygenation through chemical effects of oxygen and carbon dioxide partial pressures. This understanding should allow development of dynamic models of these interrelationships, and permit prediction of effects of hypoxia in varied situations.

The task has direct relationships to human activity in closed space station, spacecraft or submersibles, in aviation and high altitude exposures, in clinical medical emergencies on Earth or in space, and in fire extinguishment without atmospheric contamination by chemical agents. Impacts and benefits of this research and technology for the common man relate to improved respiratory support procedures in serious disease, to safety at work in hazardous closed spaces, and to environmental ozone layer protection.

Environmental Biomedical Research Data Center

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Funding:

UPN/Project Identification: 199-70-27-14

Solicitation: 95-OLMSA-01

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$196,200

Task Description:

The Environmental Biomedical Research Data Center is an established system of 30 years duration of analytic predictive modeling of human environmental stress responses, development of basic and applied research records databases, operational life-support databases, research applications, and technical documentation functions. The objective of the continuing activity is an active correlation of long-range NASA Life Sciences aerospace research functions with parallel results of multi-year undersea biomedical/bioengineering research and operational applications. The desired objective of combining relevant undersea, atmospheric, and aerospace biomedical research into its inevitable continua has special importance in predicting human and other biologic adaptations, deteriorations and residual effects in long-term exposures to environmental stress (e.g., thermal, hyperoxic, toxicologic, physical activity, gravitational, hypoxic, hypercarbic, fatigue states, physiologic, pathologic, psychological). The further objective is the protection, facilitation of access, and continuing communication of the information and analytic assets represented by the Data Center.

The present phase of development is devoted to establishment of methods, equipment, and computer technical systems for providing open access as a prototype human physiological effects data center for integration with NASA Biomedical Archive development.

Examples of present activity include analysis and modeling of extensive data concerning physiologic and toxic effects of oxygen on human lung, brain, and other organs; continued international accumulation of data allowing predictive modeling of development, prevention, and therapy of the gas lesion diseases (diving forms, aerospace, isobaric); the analysis and modeling of dynamic acute adaptations to hypoxia and hypercarbia; and the interactions of respiratory environmental stresses and respiratory function in graded degrees of physical work in hypoxia and hyperoxia. These analytic functions of the Data Center are made possible by the expanded

availability of original physiological atmospheric and undersea research information, beyond the content of open literature.

The Environmental Biomedical Research Data Center is an established unit of the University of Pennsylvania and is directly associated with the Institute for Environmental Medicine. Its functions have received guidance from NASA Life Sciences to aid its usefulness as a Prototype Data System for NASA, concerning biomedical adverse effects and adaptations to hazardous and useful situations of human exposure to environmental stress.

Two forms of active further development have occupied the previous period and the present fiscal year. One fundamental aspect, designated the "Access Program," comprises incorporation of prior and currently accumulating biomedical data systems into expanded computer methods for facilitating access, search, and selection of information. The second aspect is the continuing performance of unique analytic activity which utilizes new and existing data systems to develop predictive models of degrees and interactions of physiological effects in relation to varied forms and degrees and durations of stressful environmental exposures. Current year "Access Program" Developments Activity has included: 1) Integration of a newly procured upgraded Network Server, installation of Network Operating System, and incorporation of multi-media capability on work stations; 2) Data Center Staff Education, Microsoft Certified Systems Engineer Training, Hypertext Mark-up Language, and Editing of Active Server Pages Environments and Visual Basic Script; and 3) External User Interface development for data retrieval, establishment of Data Center "Home Page," and development of Two-Tier User Navigation System (Hypertext Pathways and Dynamic Search Sequences).

Continued incorporation of new data system assets has included: 1) Organized and analyzed Databases associating Venous Gas Embolism Severity and Decompression Sickness Incidence with the IFEM Bubble Dynamics Predictive Index of Decompression Stress; 2) Organized and statistically analyzed human Pulmonary Oxygen Tolerance Database encompassing all existing data for rate of development of severe effects of prolonged exposure to 1.5, 2.0, 2.5, and 3.0 ATA O₂; and 3) Design and Development of Data Assets Management, Search and Access Capabilities: Continued expansion of Data Center Index within Network Data base Application; Incorporation of Microsoft Index Server as Search and Retrieval Platform; Intranet Master Catalog for Access to Indexed Data Center Assets; Image Viewer Platform for Static Asset Display; Full Text Boolean Search Engines with Hypertext Results Page; and Security for Raw Data Spreadsheets.

Current year analytic modeling developments has concentrated exclusively upon graphic and mathematical descriptive correlations of the quantitative relations between two detectable effects of inadequate decompression from air breathing at a higher pressure (i.e., Doppler monitored Venous Gas Embolism and Clinically identified Decompression Sickness). These previously unidentified relations were obtained by triplex correlation of occurrence of Venous Gas Embolism, magnitude of a theoretically based index of decompression stress, and occurrence of decompression sickness, utilizing two major databases of the Data Center. The analytic modeling development and databases will be accessible on completion of the report early in the coming year period.

This Environmental Biomedical Research Data Center has been developed to provide detailed research information concerning human exposures to severe stresses of atmospheric, aerospace, and undersea environments. The basic data shows physiological effects of many different forms of stress, in acute and sustained exposures, in rest and in working situations. Analysis of these experiment databases allows understanding of the underlying biomedical mechanisms of adverse environmental effects, and the mechanisms of beneficial adaptations and survival. The range of research applications of direct data encompasses such situations as aerospace extra-vehicular activity, extreme hydrostatic and inert gas pressures of deep undersea activity, atmospheric gas toxicity including carbon monoxide poisoning, oxygen tolerance and poisoning, physical work in hypoxic atmospheres, adaptation to increased atmospheric carbon dioxide, and effects of stressful thermal environments.

These broad opportunities provide for determining degrees and limits of human physiological capabilities as these relate to normal working and to extreme emergencies. They bridge the scope of normal human endeavor in health and provide understanding of stresses in physiological derangements associated with illness. Benefits have derived in opening large undersea regions to constructive human work and in advancing safety in aerospace

operation. Other applications have provided methods for fire extinguishment which produce no environmental hazard.

Remediation of Biofilms Formed by Bacteria Isolated from Spacecraft Water Systems

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-04-11-43
Initial Funding Date: not available
Students Funded Under Research: 0
FY 1997 Funding: \$ 152,512

Solicitation: 95-OLMSA-01
Expiration: not available
Post-Doctoral Associates: 0

Task Description:

Potential applications of this research include tests for bacterial contamination and the water quality of potable, recreational, and industrial water. This research could produce on-line, real-time monitoring tests for field, home, and industrial use that will be easy-to-use, rapid, selective, and sensitive. Furthermore, the measurement and evaluation of biofilm remediation techniques are applicable to industrial water treatment. The proposed technology development will be beneficial to NASA and will lend itself to future commercial applications, thus addressing one of the aspects of NASA's Strategic Plan regarding the transfer of technology to the private sector.

Spaceflight Effects on Microbial Susceptibility to Antibiotics

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Funding:

UPN/Project Identification: 199-04-11-20

Solicitation: 93-OLMA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$150,000

Task Description:

The growth and biological functions of microorganisms are extremely sensitive to physicochemical environmental factors. However, microbes can and do adapt quickly to changes in their environment; often by opting for alternate metabolic pathways. Gravity is an important, omnipresent environmental factor in microbial growth; any alteration in gravity could be expected to affect the metabolic activity of cells. Some preliminary results from previous space flights indicate that microbial growth and susceptibility to selected antimicrobial agents are influenced by space flight. However, these results are somewhat contradictory, and the limited number of microbial strains and antimicrobial agents used in past space experiments mandates further research. The hypothesis to be tested here is that space flight will increase microbial resistance to antibiotics. The increased resistance to antibiotics may be caused by modification of cell physiology, cell structure, or the mode of action of the antimicrobial agent. Furthermore, any changes in microbial reaction to antibiotics during space flight may influence decisions on the management of infectious disease during long-duration missions. To evaluate space-flight-induced changes in antimicrobial-susceptibility patterns, we propose an experimental protocol that emphasizes a minimum of crew time, space, and equipment requirements on the spacecraft. We shall determine the minimum inhibitory concentrations (MIC) of selected microbes against several commonly used antibiotics. A test device (the Vitek antibiotic susceptibility test card) containing dilutions of antibiotics will be inoculated before launch with the test organisms and immediately refrigerated at 4°C until on-orbit incubation is begun. These credit-card-sized cards will be specially prepared to allow rapid, simple assessment of the presence or absence of microbial growth by the astronauts during flight. Microbial growth (or resistance to an antibiotic) will be apparent from a distinct color change in the test wells. The lack of microbial growth (or susceptibility to the antibiotic) will be evidenced by absence of a color change. Variance between in-flight and ground-control MIC values may reflect the effect of microgravity on actively metabolizing microorganisms.

The development of the manual card reading system for antibiotic susceptibility testing was initiated to perform such testing in the space flight environment. Such technology will allow us to answer a very important question regarding the appropriate antimicrobial therapy in space. Severe constraints on power, weight and volume, maintenance, calibration, and others limit technology available for use in spacecraft. Even though the effort was undertaken to address a specific question for space flight, this technology could be valuable for some Earth applications. For example, this technology could be used in remote settings without access to a

comprehensive diagnostic microbiology laboratory. For example, submarines, battlefield, rural areas throughout the world. The automated instrument marketed to perform the antimicrobial testing using the Vitek cards costs about \$100 K (it also performs additional functions). In addition, basic findings of the mechanism of action of antimicrobials on human microbial pathogens in space may lead to important breakthroughs to new antimicrobials on Earth. The observed changes in antibiotic susceptibility in space may provide mechanistic insight regarding microbes ability to "combat" antibiotics. The emergence of antibiotic resistance among bacterial pathogens is creating a crisis in public health and has been written about extensively in the lay press. Learning how microbes become more resistant to antibiotics in space may lead to a better understanding of the Earth-bound phenomenon of multiple drug resistant strains of human pathogenic bacteria.

The Effects of Exercise-Enhanced Denitrogenation on Altitude Decompression Sickness (DCS) Protection

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Funding:

UPN/Project Identification: 199-04-17-12

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 126,000

Task Description:

Findings from a previous study show that beginning a one-hour prebreathe profile with a 10-minute period of strenuous exercise offers a surprisingly strong and statistically significant advantage over a 1-h resting prebreathe in the prevention of decompression sickness (DCS) during a subsequent simulated extra-vehicular activity (EVA) exposure (Webb et al., 1996). This research will build on the earlier work to 1) confirm and expand the hypothesis that the benefits of the exercise-induced denitrogenation outweigh any predisposing effects of bubble nuclei formation, and 2) determine the optimum combination of parameters that result in the most effective prebreathing schedules. The experiments are expected to show improvement of as much as 50% in the denitrogenation efficiency above that seen in the earlier work. Results from this effort should provide information in support of more efficient prebreathe and EVA procedures.

Human subject exposures have been completed at the planned rate of 5 per month. Of the 180 planned exposures, 73% (132) have been accomplished as of September 30, 1997. Approximately 42% DCS incidence occurred during a four-hour simulated EVA exposure following a supine, resting 4-hour preoxygenation. This level of DCS following such a long prebreathe is double (N.S.; Chi Square = 3.15) the incidence reported during studies described by NASA in 1984. The 15 min of exercise during 90 min of preoxygenation did not show the expected improvement in DCS protection versus the previously-reported protection from use of 10 min of exercise during 60 min of preoxygenation. The current progress appears to be on track for timely completion, although difficulty in acquiring female subjects is slowing progress. Future work will continue to build on the subject-exposures already accomplished to enable better statistical analysis.

The research funded under this task is directed at preventing a high-altitude and space human health malady: decompression sickness. The task is oriented at providing a preventive protocol which is more efficient than the current method of prevention (i.e., more time and cost effective). The work has some potential for providing a better understanding of the denitrogenation process in the human body and explaining potential differences between that process under or without the force of gravity. The impact and benefits of results on humans would only be to potentially provide a more efficient procedure to prepare for extravehicular activity, thereby reducing the time needed to build the International Space Station (ISS). This could, in effect, allow the benefit of ISS research to become realized more quickly and at less cost.

FY97 Publications, Presentations, and Other Accomplishments:

Pilmanis, A.A., Krause, K.M., and Webb, J.T. "Optimizing the physiological concepts for future extravehicular (EVA) operations." Reusable Launch Vehicle Human in Space Workshop Proceedings (1997).

Webb, J.T. and Pilmanis, A.A. "The role of oxygen in decompression sickness (DCS) - Principles." Presentation at Aerospace Medical Association Meeting (1997).

Webb, J.T. and Pilmanis, A.A. "A new preoxygenation procedure for extravehicular activity (EVA)." 12th Man in Space Symposium: The future of humans in space. Proceedings (1997).

Biophysical, Mathematical Models of Gas Phase Formation

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Funding:

UPN/Project Identification: 199-70-31-20

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 2

FY 1997 Funding: \$74,000

Responsible NASA Center: Johnson Space Center

Task Description:

The calculation system presently employed by NASA for the calculation of decompression methods to avoid decompression sickness (DCS) is based upon the ratio of dissolved nitrogen in one half-time compartment to the ambient pressure. This system does not include time under reduced pressure during which nitrogen is being lost. It is founded solely upon statistical grounds, not necessarily with particular basis in biophysical principles. This system is termed the R-value approach, and needs to be modified since it is not time-dependent. To increase options in NASA mission planning and also to reduce the possible incidence of DCS in space operations, while at the same time maintaining efficiency of operations, it could be valuable to employ a staged decompression regimen which will entail a reduction of suit pressure. We will analyze extensive current laboratory altitude decompression regimens which will allow us to calculate base models. These will be incorporated into two different models which will allow us to calculate time dependent decompression tables for use in NASA EVA operations. We desire to determine practical solutions to problems involving improving the efficiency of decompression in space. The two models are: (1) the tissue bubble dynamics model of Michael Gernhardt, Ph.D. which employs the same model parameters in several tissue halftimes and a diffusional unstirred boundary layer around the free gas phase; and (2) the tissue bubble dynamics model of Wayne Gerth, Ph.D. which uses a tissue bulk diffusion term, and it varies the model parameters with only three tissue halftimes.

We will evaluate, reparameterize for hypobaric conditions, and refine two decompression models incorporating tissue bubble growth dynamics by analyzing an expanded altitude decompression database. These models and their parameters will be evaluated by increasing their ability to predict the occurrence of both DCS symptoms and venous gas bubbles (VGB) associated with the existing altitude decompression data base. These models will also be refined to assess laboratory data relating to tissue micro nuclei depletion in hypokinetic and adynamic individuals. With the addition of the metabolic gases and the redefinition of parameters, a better accordance with decompression data (both DCS and gas bubbles) will occur than with the current R-value method. This will concern both DCS symptoms and Doppler detectable gas bubbles.

Two models have been developed that describe the growth of a gas phase during depressurization, a situation that can lead to decompression sickness. In both models, dissolved inert gas enters and leaves a tissue by perfusion.

To describe the incidence or risk of decompression sickness, the models use a function that relates the growth of a gas phase to the probability of developing decompression sickness. These models incorporate the concept that there exists *in vivo* a population of microbubbles of a critical radius or greater. The distribution is described by a power law function.

These microbubbles are generated by musculoskeletal activity and resolve (dissolve) during inactivity or while in zero-g. The gas bubble growth for any radius is given by an equation which incorporates diffusion constants, gas solubility, bubble radius, and shell thickness. These new models will be used to characterize the depressurization characteristic of a number of scenarios in space when the bubble distribution has been added.

This work describes a process that affects individuals on Earth as well as astronauts. It will lead to the development of new types of decompression tables that will mitigate decompression sickness and can be applied to Earth-based decompressions as well. The work has described a new and rational basic understanding of the cellular mechanisms behind decompression sickness.

This information would also be of value for both SCUBA and commercial divers, especially those involved in the recovery of deep-sea oil. The benefits to commercial diving should be substantial by the implementation of certain aspects of this work.

FY97 Publications, Presentations, and Other Accomplishments:

Dervay, J., Powell, M.R., and Fife, C.E. "Effective lifetimes of tissue micronuclei generated by musculoskeletal stress." Aerospace Medical Society Annual Meeting (1997).

Foster, P.P., Conkin, J., and Powell, M.R. "Onset and recovery of venous gas emboli from each limb and association with hypobaric decompression." Undersea and Hyperbaric Medical Society Annual Meeting (1997).

Loftin, K.C., Conkin, J., and Powell, M.R. "Modeling the effects of exercise during 100% oxygen prebreathe on the risk of hypobaric decompression sickness." Aviat. Space Environ. Med., 68, 199-204 (1997).

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Factors Affecting Decompression Sickness in Astronauts During Extravehicular Activity

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

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Task Description:

Decompression sickness (DCS) has not been reported during extravehicular activity (EVA), but ground-based experiments indicate a 20-30% incidence of pain and 2-3% incidence of chokes or cerebral symptoms. While incomplete reporting of DCS during EVA cannot be ruled out, DCS risk may be influenced by environmental and physiological factors, some of which are associated with microgravity and which differ from conditions prevailing in ground-based studies. This hypothesis is the basis of our experiments in which we emphasize exercise and terrestrial simulations of microgravity. We measure respiratory nitrogen elimination during 2.5 or 3.5 hrs of preflight oxygen breathing and monitor subjects for precordial Doppler bubbles during 4 hr exposures at 30,000 feet after ascents at 1,000 or 3,500 ft/min. We investigate the effects on respiratory nitrogen elimination and DCS risk of physiological and environmental factors that may affect nitrogen exchange or bubble formation in the body. To date, the overall DCS incidence is about one third in 213 studies. Analysis of nitrogen elimination data by multiple regression and of DCS and Doppler bubble data by logistic regression indicates that exercise during oxygen prebreathe, prebreathe duration, and immersion during prebreathe significantly enhances nitrogen elimination and reduces the incidence of bubbles and DCS. Body weight and terrestrial gravity also appear to be risk factors for DCS suggesting that DCS risk may be inherently lower in microgravity than at 1-G due to adaptations which influence both bubble formation and tissue perfusion. Our studies in the next year will apply our findings to EVA during construction of the space station.

EVA from the space shuttle requires decompression from a cabin pressure of 14.7 psia to a suit pressure of 4.3 psia. To achieve this without excessive risk of DCS, shuttle astronauts usually perform a multi-hour decompression stage at 10.2 psia before further decompression to 4.3 psia. (In 53 EVAs through 1995, the mean stage time was 36.7 hrs. In an alternative pre-EVA procedure, astronauts breathe oxygen at 14.7 psia for 4.5 hrs and decompress directly to 4.3 psia.) Stage decompression will be inconvenient during construction of the space station starting in 1999, however, and a pre-EVA period of 1-3 hours is desired.

DCS has not been reported during EVA, but ground-based simulations have resulted in 20-30% DCS incidences. Our work suggests that gravity contributes to this discrepancy and that pre-EVA periods may be possible that are far shorter than those presently used (Vann and Gerth, 1997). Further reductions in pre-EVA time may be achievable, moreover, by taking advantage of our finding that exercise during oxygen prebreathing also reduces DCS risk (Vann and Gerth, 1997). These hypotheses were supported by our probabilistic modeling program (Gerth and Vann, 1997).

During the past year, we proposed to the Astronaut Office (JSC/CB/XA) that microgravity simulation and prebreathe exercise be used in experimental tests of pre-EVA procedures that might be employed during space station construction (Vann and Gerth, 1997). This proposal has been incorporated into a multi-center trial which will begin in November 1997. The decompression trials we were to conduct during the past year have been deferred and will take place as part of the space station tests.

DCS occurs as a result of hyperbaric exposure (diving, compressed air work, hyperbaric medicine) and hypobaric exposure (air or space travel). DCS mechanisms involve the exchange of inert gases and the formation of bubbles in the body. Although the same physiological mechanisms are involved in DCS that result from either hyperbaric or hypobaric exposure, the environmental and physiological conditions can be different and can lead to dissimilar medical outcomes and risks. For example, our experimental results suggest that humans are less susceptible to altitude DCS in space than on Earth because of naturally occurring adaptations to microgravity. Similar observations apply to the effects of immersion in diving (a terrestrial simulation of microgravity) when compared to hyperbaric exposure under dry conditions where gravity effects are present. Our goals are to investigate the roles of physiological and environmental factors that affect inert gas exchange and bubble formation and to develop a comprehensive mathematical description of these effects. An understanding of the fundamental nature of decompression and a mathematical model describing the kinetics of DCS probability will improve the safety and efficiency with which humans can live and work in the useful range of barometric pressures.

FY97 Publications, Presentations, and Other Accomplishments:

Gerth, W.A. and Vann, R.D. "Probabilistic gas and bubble dynamics models of altitude decompression sickness." Workshop on EVA Prebreathe Reduction, Center for Advanced Space Studies, Houston (January 27 - 29, 1997).

Vann, R.D. and Gerth, W.A. "Is the risk of DCS in microgravity less than on Earth?" (Abstract) *Aviat. Space Environ. Med.*, 68 (7), 621, Abstract 45 (1997).

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Vann, R.D. and Gerth, W.A. "The effect of prebreathe exercise on DCS risk." Workshop on EVA Prebreathe Reduction, Center for Advanced Space Studies, Houston (January 27 - 29, 1997).

Molecular Damage of Human Cells by X-rays and Neutrons

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Funding:

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Initial Funding Date: 1995 Expiration: 1998
Students Funded Under Research: 2 Post-Doctoral Associates: 5
FY 1997 Funding: \$220,538
Joint Agency Participation: DoD

Task Description:

The central goal of this standard ground-based research project is to identify DNA damage induced by low- and high-LET radiations at moderate doses. Damage induced by fission neutrons and by 1 GeV/n Fe ions will be compared to that induced by isoeffective effective X-ray doses in human immortalized cells originating from different tissues. Recent data suggest that exposure to ionizing radiation results in genetic instability, which is expressed as an increase in the levels of transforming, mutagenic, and cytogenetic damage many cell generations after radiation exposure. Our working hypothesis is that radiation response principally occurs in the early stages of carcinogenesis or other radiation-induced pathologies. In the cellular models proposed here, this early phase has a duration of about 10-12 cell divisions, or about 2 weeks. We will study radiation-induced genetic alterations at several time points within the first 2 weeks post-irradiation using several established or novel techniques presently used in our laboratories for studying molecular changes associated with human malignant disease. Accordingly, we will assess persistent alterations in the expression levels of both known and novel gene transcripts, using novel high-throughput tools for panoramic profiling gene expression in irradiated versus control cells and a direct analysis of transcripts by Northern blotting. Additional types of DNA damage to be studied include induced changes in several known cancer-related genes. In the proposed studies, reactor-produced neutrons and HZE Fe ions are important components of the space radiation environment. A sizeable proportion of the dose behind spacecraft or lunar shielding is due to neutrons. Neutron irradiations will be performed at the TRIGA Reactor Facility of the Armed Forces Radiobiology Research Institute (AFRRI) in Bethesda, Maryland, while Fe ion irradiations will be provided at the AGS facility at Brookhaven National Laboratory (BNL).

The overall objective of our program is to characterize in molecular terms genetic alterations induced by ionizing radiation. Our FY97 specific aims in pursuit of this goal included: (1) performing our first experiments at BNL, obtaining a radiobiological characterization of the Fe ion beam using our test systems; (2) refining and applying our novel differential gene expression assay to acquire a database detailing determination, identification, and characterization of genes differentially expressed by high- and low-LET radiation; and (3) continuing studies of site-specific genomic instabilities with special attention to mismatch repair mutations induced by ionizing radiation.

The overall objective of our program is to test the hypothesis that residual damage remaining in cells offers the best opportunity to observe a molecular radiation signature. We further hypothesize that a molecular signature from high-LET radiation will be different from a molecular signature from low-LET radiation. To investigate these hypotheses, we have used X-rays, fission neutrons, and 1 GeV Fe ions to investigate cellular responses at various times up to 2 weeks post-irradiation. Molecular endpoints have included mRNA expression levels, as well as the induction of microsatellite mutations.

The aim of our gene expression studies is to identify genes involved in radiation response at the cellular level, including neoplastic transformation. To accomplish this objective, we applied an advanced high-throughput tool for gene expression analysis, now called BIGEL. This method utilizes a random gene selection and parallel array format. The BIGEL array reveals the panorama of changes in expression of 192 genes/array providing insights into how gene expression results in a complex phenotype.

Summary of FY97 work accomplished/in-progress using BIGEL:

Libraries:

- 1) HL60 undifferentiated cell line cDNA library in Lambda ZAP II cloning vector;
- 2) ZR-75-1 breast carcinoma 5'-stretch cDNA library in Lambda gt10 cloning vector.

Overall cDNA-library screening results:

Total cDNA screened: 5,600

Number of differentially expressed cDNAs: 111

Number of differentially expressed clones identified & confirmed: 61 (55%)

To summarize experiments performed, approximately 5,600 cDNA were arrayed on 28 exact duplicate membranes. Each pair of arrays was probed with total cDNA derived from irradiated versus control cells. The conditions for studying differential gene expression were: [Library (number of clones screened per condition, number differentially expressed cDNAs/cDNAs identified), experimental cell line, radiation type, radiation dose, sampling time after initial exposure]: 1(2000, 27/17), HL60 cells, X-rays, 20 Gy, 3 h; 1(1000, 50/20), MCF-7 cells, fission neutrons, 1.2 Gy, 7 d; 1 (1600, 34/21), MCF-7 cells, X-rays, 5 Gy, 7 d; 2 (1000, 3/3), MCF-7, fission neutrons, 1.2 Gy, 7 d.

As an initial demonstration of our approach, BIGEL was used to characterize immediate early gene expression 3 h after HL60 cell exposure to 20 Gy of X-rays. The discovery of a first novel radiation responsive gene using BIGEL has been described in "FY96 Task Progress;" we recently published some of these findings in *Oncogene*. Other results can be summarized as follows. We determined partial DNA sequences of all 17 cDNAs and compared them with other known sequences using the GenBank/EMBL database. Tentative GenBank matches were identified for 16 isolated cDNAs, 2 corresponded to expressed sequence tags (ESTs) and 14 corresponded to known genes. A sequence one cDNA clone (C42) did not correspond to any known GenBank entries; it may represent a novel transcript. Northern blotting of this gene identified an mRNA of 1.9 kb; the PCR product insert size was 0.9 kb. Our paper, recently submitted to *Cancer Research*, describes 14 novel immediate early radiation responsive genes.

Consistent with the approved research plan, we have focussed on a discovery and characterization of genes associated with late response after X-ray or fission-neutron irradiation. We used isosurvival doses of 5 Gy or 1.2 Gy in experiments with X-rays or fission neutrons, respectively. Of 3600 cDNA clones screened by BIGEL using materials derived from fission neutron- or X-irradiated cells, 50 or 34 clones, respectively, were found to be differentially expressed. What are the identities of the differentially-expressed late genes? Of the 84

differentially expressed transcripts, 44 transcripts were tentatively identified through database comparisons. Of 44 identifications, 39 were of known genes and 5 of potentially novel genes. A relative simple picture has emerged from these data. The majority of genes found to be dysregulated 7 d after fission neutron- or X-irradiation are known to be important for the normal physiology or cellular architecture. For example, gene products associated with protein synthesis (including seven ribosomal proteins, two elongation factors, two mitochondrial oxidases, and three genes involved in glycolysis) were observed to be down-regulated in progeny of irradiated cells, compared to control cells, suggesting a sustained genetic damage in cells that initially survived radiation-induced injury.

Summary of FY97 work accomplished/in progress using other gene expression profiling methods:

In addition to BIGEL, the late radiation damage was surveyed using another tool for gene expression analysis, ATLAS (from Clontech). Both approaches use a similar parallel array format. In contrast to BIGEL, ATLAS expression cDNA array reveals changes in expression patterns of 588 known genes that are under tight transcriptional control; these genes are organized on duplicate membranes into six functional classes that represent many current areas of radiobiology research. In our application, ATLAS has been used to predict possible classes of differentially expressed genes, rather than to search for changes in expression of individual genes. Using the ATLAS approach, 19 out of 588 cDNAs were found to be highly expressed in control cells, including three ribosomal proteins, three transcription factors (*c-myc*, *Waf1/Cip1*, *rho*), two heat shock protein, five kinases, and six DNA binding proteins. Only one gene, encoding a retinoic acid receptor II, was found to be altered 7 d after 1.2 Gy of fission neutrons. These findings suggest an epigenetic component of radiation carcinogenesis, but additional studies are needed to fully assess the biological significance of this result.

We characterized in terms of kinetics several known radiation-inducible genes, including p53-regulated genes (*Waf1*, *gadd45*, *mdm2*) as well as *proliferating cell nuclear antigen (PCNA)* and several oncogenes, including *c-fos* and the *early growth response-1 (egr-1)* gene. These data suggest a complex transcription pattern of genes involved in the regulation of specific cellular responses following radiation exposure, including compensatory cell proliferation and repair.

Finally, we have begun a detail characterization of *pS2* coding for trefoil peptides that function in tissue repair and inhibition of proliferation; thus, high levels of *pS2* appear to be necessary for protection against injury to many tissues, including normal breast and gastrointestinal tract. Our experiments have identified *pS2*, for the first time, as a new radiation-responsive gene which is up-regulated in irradiated MCF-7 WT breast carcinoma cells. Interesting, *pS2* expression was observed to be delayed for 1 d or 4 d after exposure to fission neutrons or X-rays, respectively. Thus, the up-regulation of *pS2* appears to be both time- and radiation quality-dependent. We plan to examine *pS2* kinetics in MCF-7 WT cell samples irradiated with 1 GeV/n and 0.6 GeV/n iron ions. We feel that *pS2* may emerge as an important novel radiation-type-specific late marker of tissue injury/repair. Additional experiments, currently in progress, have been designed to answer whether this delayed response also occurs *in vivo*. Due to practical difficulties, these studies are at present limited to using mice as a model system and investigating the effects of X-irradiation. To date, we have performed two experiments, using 10 animals per experiment, at 7.5 Gy. Multiple-tissue RNAs has been extracted from all the main organs, except skin and nervous system. A mouse-specific *pS2* probe has been generated based on the published DNA sequence data. Sampling time-points are 4 d, 7 d, 14 d and 21 d. Four-day data available suggest a tissue-specificity.

Summary of FY97 work accomplished using microsatellite assay:

We have investigated the possibility that ionizing radiation can induce deletions in short nucleotide repeat sequences known as microsatellites. Such mutations are sometimes associated with defective mismatch repair and many diseases including cancer could affect mismatch repair. Assuming that moderate to high doses of radiation result in detectable yields of such mutations, we subjected numerous aliquots of DNA from control and irradiated cells to polymerase chain reaction (PCR) amplification using primer sets for loci containing microsatellite sequences. The investigated loci were D2S123 containing (CA)_n, BAT26 containing (A)_n and

located in intron 5 of the human *msh2* mismatch repair gene, and BAT40 and located in an intron of the *3- β -hydroxysteroid dehydrogenase* gene. The DNA in each 10- μ l PCR aliquot was diluted to picogram levels, equivalent to the DNA contained in only several cells, so that an alteration in only one of the several cells might be detected. As in studies described above, human pre-leukemic HL60 and human breast carcinoma MCF7 WT cells were irradiated with X rays or fission neutrons. Results were negative for D2S123 and BAT40. Frequent BAT26 alterations were observed after 20 Gy of X-irradiation or 1.2 Gy of fission neutron irradiation.

We have applied a method originally developed for cancer research to the detection of radiation-induced alterations in specified DNA sequences. This method provides an alternative to typical methods requiring clonal selection and expansion. A practical advantage is the flexibility in selecting the sampling time for DNA analyses.

In these preliminary studies, use of a high dose of radiation followed by early assay for mutation was designed to produce a high mutation yield. The same techniques can be applied for longer post-irradiation times and lower doses to study mutation rates in surviving cells and radiation-induced genomic instability. Results for 2 loci on chromosome 2 (D2S123 and BAT40) were negative following 20 Gy of X-irradiation, although it must be pointed out that the sample sizes were small. The positive results for BAT26 suggest a specific mutation hot spot induced by ionizing radiation in the human *msh2* gene, suggesting that mutator pathways are involved in radiation response. Alterations occurred only in BAT26, approximately 20% of which is a poly-A sequence, so that the observed alterations may not necessarily be a microsatellite mutation. This line of investigation has been abandoned due in part to the unanticipated very high cost of the assay in terms materials, time, and effort.

This research seeks to understand at the molecular level the biological consequences of exposure to an increased radioactive background. Recent investigations demonstrated that A-bomb survivors had increased rates of chromosomal aberrations (reciprocal translocations), somatic mutations, an elevated risk of leukemia, and breast and lung tumors. Also, studies carried out in areas contaminated after the Chernobyl disaster allow for definite conclusions that complex changes take place in animal and man in an altered radioecological situation. These changes include disorders in the immune and hemopoietic systems, the gastrointestinal tract, development of atherosclerosis, increase of leukemia and thyroid gland cancers, and premature aging of the immune system as well as the whole organism. A specific point we attempt to resolve by performing these studies concerns the ability to factor out a radiogenic contribution to health effects from contributions resulting from complex interactions between radiation and other harmful agents to which a person is exposed. On Earth, these interactions could be between radiation and harmful chemicals (heavy metals, nitrites, pesticides, free-radical generators, etc.). In space, these interactions could be between various types of radiations and microgravity. In these scenarios, radiation could play either a major role at certain stages of pathology (for example, in the early stages of radiogenic cancer development) or it could play a minute role by itself but enhance the effect of other agents. Our studies are important since they will delineate the expression and mutation profile of specific genes that are differentially regulated in radiogenic cancer and other late effects of radiation. One potential advantage emerging from our studies is the possibility of modeling radiation-specific effects under controlled laboratory conditions. Persons on Earth and in space are exposed to radiation environments whose radiation quality could differ; specific examples here are astronauts exposed to both low-LET and high-LET environment, and bone marrow transplant patients exposed to low-LET radiation, or certain home dwellers exposed to high-LET radiation from radon. Therefore, our analyses of the basic molecular mechanisms underlying the complex biological phenomenon of radiation-induced cellular change could equally be applied to the question of health risk caused by ionizing radiation environment on Earth and in space.

The conversion of a normal cell into an abnormal cell is largely the result of change in gene expression patterns between to the two cell types. Our studies are designed to define cellular transformation in molecular terms by characterizing the altered genetic program induced by exposure to radiation. Knowledge of radiation-specific gene damage will be most valuable for the purpose of radiation protection as well as litigations involving radiation as a causal agent of a disease. Finally, antagonists or agonists of radiation-specific gene therapy could be applied in future advanced molecular therapies or preventive strategies, such as those currently being developed for other specific human populations at risk due to genetic hereditary factors (for example, breast or colorectal cancers or mental disorders)

We developed and verified a new promising approach for comparing patterns of gene expression in experimental versus control cells. In this approach, the entire mRNA populations are hybridized to nucleic acid arrays -- a method adopted commercially for high-throughput expression analysis. However, in contrast to commercial methods such as ATLAS, our approach permits new gene discovery and analysis. In addition to investigating radiation effects, BIGEL technology has a wide range of applications such as investigating normal biological and disease processes, profiling differential gene expression, and discovering potential therapeutic and diagnostic targets, including targets for gene therapy.

FY97 Publications, Presentations, and Other Accomplishments:

Balcer-Kubiczek, E.K. "Application of an unbiased two-gel cDNA library screening method to expression monitoring of genes in irradiated versus control cells." Twelfth Man in Space Symposium, Washington, DC, abstract P07-162 (1997).

Balcer-Kubiczek, E.K., Abraham, J.M., Harrison, G.H., Han, L.-H., Zhang, X.-F., Shi, Z.-M., and Meltzer, S.J. "Application of an unbiased two-gel cDNA library screening method to expression monitoring of genes in irradiated versus control cells." Forty-fifth Radiation Research Society Annual Meeting, abstract P07-162 (1997).

Balcer-Kubiczek, E.K., Harrison, G.H., Meltzer, S.J., Han, L.-H., Zhang, X.-F., Shi, Z.-M., and Abraham, J.M. "Csa-19, a radiation-responsive human gene, identified by an unbiased two-gel cDNA library screening method in human cancer cells." *Oncogene*, 14, 3051-3057 (1997).

Balcer-Kubiczek, E.K., Meltzer, J.M., Han, L.-H., Zhang, X.-F., Harrison, G.H., and Abraham, J.M. "Novel method for detecting gene expression alterations induced by X-rays, fission neutrons and Fe-ions." Eighth Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY.

Harrison, G.H., Balcer-Kubiczek, E.K., Zhang, X.-F., Zhong, S.M., Qin, J.T., and Meltzer, S.J. "Detection of radiation-induced deletions in microsatellite loci using extreme dilute DNA PCR." Forty-fifth Radiation Research Society Annual Meeting, abstract P22-447 (1997).

Ioffe, V. and Balcer-Kubiczek, E.K. "The unbiased two-gel cDNA library screening method for detecting radiation-responsive genes." Student Research Forum, Summer Research Training Programs, the University of Maryland School of Medicine, Baltimore, MD, abstract 97-09.

HZE and Proton-Induced Microenvironment Remodeling

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$202,467

Task Description:

The cosmic radiation environment is a complex mixed radiation field. This research will contribute to an experimental design that focuses on quantitative assessment of specific tissue effects induced by important components of the space radiation environment: protons and iron ions. Determining the risk from radiation exposure during space travel is constrained by the lack of understanding of basic mechanisms of radiation effects on multicellular tissue processes that lead to functional impairment and carcinogenesis. We have identified microenvironment remodeling as a novel early endpoint of radiation exposure and have recently found certain effects that appear to be dependent on radiation quality. The microenvironment of tissues encompasses insoluble extracellular matrix proteins and soluble growth factors. Our studies in the mammary gland have demonstrated that radiation-induced microenvironment alterations are rapid (<24 hr), persistent (>7 days), and sensitive to low doses (<50 cGy). These studies will evaluate the generality of this novel tissue process and of its HZE dependence. Identification of tissue-specific remodeling will provide fundamental knowledge of radiation effects on selected tissues, generate correlations between events in tissue remodeling, determine which events are tissue-specific or radiation quality dependent, and quantitate these events for correlation with radiation fluence or dose. The specific goals of this project are to evaluate early (~15 min - 14 day) temporal and spatial changes in the composition of the irradiated microenvironment as a function of tissue type, radiation quality and dose, or particle fluence. Liver, skin, Harderian gland, and brain from animals whole body irradiated with 600 MeV and 1 GeV Fe particles, 200 MeV plateau protons or ⁶⁰Co gamma-radiation will be evaluated. Selected changes will be quantified using image analysis and dose response relationships will be determined, with an emphasis on doses of less than 1 Gy. Understanding the mechanisms of tissue response will contribute to risk assessment and may lead to new strategies for intervention.

Tissues (mammary gland, skin, and liver) were acquired from animals irradiated with 1 GeV iron particles at the AGS at Brookhaven National Laboratory in October 1996. We have used the mammary gland as a model for developing immunolocalization techniques applicable to skin and liver in the next year of study. Transforming growth factor-beta (TGF- β), tissue plasminogen activator (tPA), laminin, fibroblast growth factor-2 (bFGF), and plasminogen activator inhibitor-1 (PAI) were mapped in murine mammary gland using dual and triple fluorescence labeling and time points of 1hr (0 and 160 cGy) and 12 hrs (0, 3, 10, 40, 80, 160 cGy) were

surveyed. Our focus has been to define either image- or biologically-based parameters that inform us to the frequency, character or mechanism of laminin disruptions.

A new avenue of investigation is based on literature showing that fragments of laminin induced the expression of tPA, which in turn augments degradation of laminin. Since tPA is also transcriptionally induced by irradiation, this type of response could perpetuate and aggravate degradation of laminin following irradiation. We asked whether antibody localization of tPA coincided with degradation of laminin in irradiated mammary gland. We developed a dual immunostaining protocol that allows simultaneous determination of their expression. We found that the basement membrane is strongly stained with tPA suggesting that it is a reservoir with potential for mediating laminin degradation in mammary gland. Similar studies will be conducted in liver and skin samples.

Protocols for large-scale data acquisition using two and three channel digital imaging and analysis were established and tested. Image analysis is being performed in a two-step process. The first step consists of a visual survey of 50-70 scaled and/or false-colored (color assigned as a function of intensity) images presented as a function of dose in an ordered contact sheet format. These ordered contact sheets are generated for each channel (red, green, and blue fluorescence, respectively) and full color (rgb color). Using this approach, quantifiable changes in antigen expression or distribution can be rapidly identified. All ordered contact sheets and all the full resolution scaled images are available to collaborators via the LBNL mass storage system (MSS) and the LBNL digital image library (ImgLib). The generation of ordered contact sheets allows us to rapidly survey a large matrix of time points, doses, and antigen combinations so that the magnitude of changes and heterogeneity can be assessed. Based on this information, we determine the number of images required in each region of the tissue for proper quantification and statistical analysis.

The following is an example of how changes in the amount and distribution of an antigen are being identified and quantified. An initial survey (using ordered contact sheets) of latency associated peptide (LAP) immunolocalization as a function of dose indicated that significant changes were occurring in the AS. For LAP in unirradiated tissue, a dim, incomplete, and thin outline of a lacy network of LAP immunofluorescence existed. At 12hr after 160cGy exposure, the immunofluorescence was brighter and the lacy network became thicker with a more complete and filled out structure. Based on this observation, we designed an image analysis protocol that could quantify this effect. Using the software package ScilImage, images were background corrected and regions of epithelium were edited from the image. Next, a set intensity threshold for image segmentation was established using images of irradiated tissue. For irradiated tissue, the resulting binary images showed a high proportion of pixels above the threshold and a relatively complete AS network was observed. The sum of all the pixel intensities in this region of interest was then divided by the total area examined. The same intensity threshold was used to segment background corrected images of unirradiated tissue. In this case, a lower proportion of pixels were above the threshold. As with irradiated tissue, the sum of all the pixel intensities in this ROI was divided by the total area examined. A clear quantifiable difference was seen between irradiated and non-irradiated tissue with only a total of 8 images.

Our current efforts focus on applying this approach to skin and liver in order to map the radiation induced protein expression for comparison to the mammary gland. We will classify radiation-induced changes in protein localization as tissue dependent, dose dependent, and LET dependent with the goal of defining underlying mechanisms of the biological consequences of particle traversal.

Factors that influence neoplastic transformation, the escape from normal regulatory controls, and the rate of progression of the initiated cell *in vivo* are currently poorly understood. Our basic research is directed towards an understanding of the factors that influence the progression of neoplastic disease. New knowledge of the critical role that microenvironment plays in eliciting appropriate function in normal cells is the basis for targeting radiation-induced microenvironment alterations for understanding how the microenvironment in which the initiated cell finds itself can dramatically influence its ability to express the altered phenotype. By studying different radiation qualities, which as evidenced by our preliminary studies elicit different types of microenvironments, we can further refine our understanding of how microenvironments affect the development of

cancer. A potential benefit of this research is the possibility designing therapeutic interventions to interrupt this process before frank malignancy is evident.

Lens Epithelium and Proton-Induced Cataractogenesis

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Funding:

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Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$204,612

Task Description:

Cataracts are a potential late side-effect of space travel that impacts risk assessment and spacecraft design. Presently, there are inadequate data to estimate accurately the risk of radiation-induced cataract in man at the relatively low particle exposures anticipated for space flight. Cataracts also arise in uveal melanoma patients as a complication following their successful treatment with proton or helium radiotherapy. We have been studying the relationship between the calculated helium-ion exposure of specific sublenticular volumes and the later appearance and location of cataracts. The objective of the proposed research is to determine the proton-induced alterations in chromosomes and in protein expression that are important to cataractogenesis, and ultimately to develop strategies to diminish the incidence or severity of these changes. We will test a hypothesis that radiation-induced changes in protein expression are important in cataract formation. To this end, experiments have been designed with three specific aims: 1) Characterize the acute radiation response of cultured human cells of the lens epithelium grown on extracellular matrix. Quantitative dose-response measurements between proton- and x-ray-induced survival, and yields of micronuclei and chromatin breaks and rejoining will be determined. The fidelity of chromatin repair will also be assessed; 2) Radiation is known to induce basic Fibroblast Growth Factor (bFGF) in cultured endothelial cells and this cytokine is associated with changes in the radiation response. Experiments are proposed that will determine whether protons or x-rays change levels of bFGF mRNA or protein in the lens epithelial cells; and 3) An investigation of the possible modification of intracellular lens proteins by protons is proposed. The proposed work will elucidate relationships between proton-induced damage to the chromatin of lens epithelium *in vitro*, and biological consequences to the cells surviving the resulting damage. This knowledge will allow correlative comparisons with available experimental work *in vivo*, and may provide a basis for elucidating the biological mechanisms contributing to cataractogenesis and to improved approaches to estimate risk of cataract due to exposures in space travel.

Our major accomplishments during the past two years are: 1) Development and characterization of a novel human lens epithelial cell model cultured on a bovine-derived extracellular matrix (ECM); 2) Use of three molecular markers (γ -crystallin, p57^{KIP2} and MIP26) for demonstrating that the human lens epithelial cells in our system undergo lens fiber cell differentiation; 3) Development of assays for determining radiation-induced loss of epithelial cell viability; 4) Obtained immunofluorescent evidence for enhanced FGF-2 expression in exponentially growing HLE 6 hours after 4 Gy x-rays; 5) Development of reverse transcription/polymerase chain

reaction techniques to detect FGF-2 gene expression from small sample sizes; 6) Quantitation of FGF-2 mRNA pre- and post-irradiation with protons and x-rays is in progress with both exponentially growing and confluent HLE cells; 7) Quantitation of FGF-2 protein intracellularly and in exogenous media is in progress; 8) Characterization of proton- and x-ray-modulation of lens crystallin proteins is underway for both exponentially growing and confluent HLE cells; and 9) Initiation of experiments to measure proton and x-ray dose-dependent yields of micronuclei, chromatin breaks, and apoptosis.

The novel aspect of this work is that we have developed an *in vitro* human lens epithelial cell model that can be maintained for months and that expresses several molecular markers indicative of lens fiber cell differentiation as observed *in vivo*. We have used this model to ask specific questions about basic mechanisms of proton and x-ray radiation-induced damage that lead to the expression of late tissue damage in the form of lens opacification termed cataract. At issue is a determination of what is the target for biological damage that leads to cataract, what pathway(s) of tissue response is (are) involved, and whether counter-measures can be conceived. Species-specific differences in crystallin are known to exist which underscores the value of our human system. After two years of work we have preliminary evidence for a proton- and x-ray-induced expression of the cytokine FGF-2. This report is a novel finding for the lens, and in the two years since our original proposal there have been several reports in the literature linking FGF-2 overexpression with downregulation of normal cell attrition by apoptosis. The lens is avascular and bound by the lens capsule; it has no clear method to clear moribund cells. Only recent literature suggests that apoptosis can be demonstrated in human lens with cataract. We also have preliminary evidence for changes in crystallin protein profile after proton and x-ray irradiation. We believe the unique endpoints we have developed with this tissue model will allow us to test the hypothesis that FGF-2 is involved in proton-induced cataractogenesis, and to determine how FGF-2 expression is correlated with quantitative measures of chromatin damage in order to elucidate underlying mechanisms of action.

The lens of the eye is considered one of the critical organs in the assessment of human risk from radiation in space. It is a superficial tissue with little body shielding and demonstrates a late expression of damage in the form of lens crystallin protein opacification called cataract. During the past 10 years, the paradigm for radiation induction of cataract has focused on genomic damage of lens epithelial cells leading to altered crystallin proteins. Little progress has been made, however, in establishing the specific details of the the molecular mechanisms due to the extreme difficulty in cultivating human lens cells or tissues, and due to the limitations of studying lens tissues from other species. The species-specificity of the lens crystallin protein family is well known. Very little research therefore has been done to develop strategies to diminish the incidence or severity of radiation damage to the lens.

Since 1988, new information has become available on the radiation environment in space, and on the human experience with radiation exposures from the atomic bomb survivors. Based on this information, there is reason to consider lower career dose limits for those involved in space activities. One of the major concerns is that there are virtually no data from studies of humans for either deterministic effects or the induction of cancer by heavy ions or protons, in particular with protracted exposures. This problem contributes significant new importance to the selection of career crew exposure limits and the level of shielding required for space travel, especially into deep space. The available biological information on the particle radiation-induced cataract indicates the extent of our lack of data and has only heightened the level of uncertainty in assessing radiation risk. Some intriguing new data on the inhibition of radiation cataractogenesis by the aminoalkyl phosphorothioate analog WR-77913 provides incentive to the pursuit of cataract countermeasures, and may reveal the role of other critical targets of damage in the eye (e.g., the ciliary body) that impact the expression of lens damage.

Irradiation of the young mammalian lens causes mitotic arrest followed by apparent excess mitosis with production of fragmented nuclei and degenerate cells. Cataracts can be induced by lower doses of high linear energy transfer (LET) radiations compared to x- or g-rays. Disorganization in the meridional row and the frequency of abnormal mitoses and micronuclei are related to both the fluence (number of heavy particles/unit area) and also to the LET of the charged particles. At a given dose, as the LET of the radiation increases, the number of abnormal mitotic figures, micronuclear frequency, and disorganization of the meridional row also

increases. The severity of the meridional disorganization and micronuclei number go up with the increasing fluence or dose for particle of the same LET. Fractionation of the charged particle irradiation does not produce dose sparing, and in some cases produces a dose-dependent enhancement in the incidence of cataract. These data support a generally accepted hypothesis that radiation cataractogenesis is the result of genomic injury to the lens epithelial cells. Analysis of the occurrence of posterior lenticular cataracts in patients treated with low-LET radiation for cataracts had in the past led to the commonly accepted threshold dose of 2 Gy for cataract induced by a single acute exposure. A new technical report has been published that reexamines the incidence of cataracts seen in the years 1949-64 among 2249 Hiroshima atomic-bomb survivors with known Dosimetry System 1986 (DS 86) doses. Among several dose-response relationships with or without two thresholds, the best fit based on binomial odds-regression models is achieved with a linear-linear dose-response relationship that assumes different thresholds for neutrons and gamma-rays. The estimates of the two thresholds differ significantly from zero, but both are much less than the accepted dose threshold of 2 Gy.

We are studying human lens epithelial cells *in vitro* for the purpose of determining what specific proton-ion-induced alterations in chromosomes and in protein expression are important to cataractogenesis, to develop strategies to diminish the incidence or severity of these changes, and to provide quantitative information on the risk of cataract from exposure to protons. In particular, we are examining two alternative mechanisms of cataractogenesis involving radiation-induction of basic bFGF in human lens epithelial cells functioning either to alter the normal program of crystallin expression and thereby disrupting normal fiber formation, or the radiation-induced bFGF acting to hinder cell loss processes which leads to the formation of aberrant lens fiber formation. This task assumes that the risk of radiation-induced cataract to man in space is the same as the risk to man of radiation-induced cataract on Earth. The effects of microgravity and other stressors from space flight on susceptibility to radiation-induced cataract have not been investigated. The impact of a successful determination of the basic molecular and cellular mechanisms underlying radiation-induced cataract may aid in devising countermeasures to avoid the risk where possible in medical treatments or occupational exposures. Potential benefits to be gained by the development of the proposed research plan include a more realistic estimate of the risk of radiation-induced cataract that could impact the design of payload requirements or operational limitations including extra-vehicular activity for flight missions.

FY97 Publications, Presentations, and Other Accomplishments:

Blakely, E.A., Bjornstad, K.A., Chang, P.Y., Aragon, G., and Lin, S.P. "Radiation effects on the balance between cell growth, differentiation and death in human lens epithelial cells." (Abstract) Eighth Annual Investigators' Workshop in Space Radiation Research, Brookhaven National Laboratory, Upton, Long Island, NY (April 29 - May 3, 1997).

Blakely, E.A., Bjornstad, K.A., Chang, P.Y., Kaur, B.S., Aragon, G., Lin, S.P., Lui, G.M., and Polansky, J.R. "Radiation response of a transformed human lens epithelial cell line to x-rays, 55 MeV Protons and 32 MeV helium ions." (Abstract) 45th Annual Meeting of the Radiation Research Society, Providence, RI (May 3 - 7, 1997).

Proton Radiation Studies

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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

In 1963, NASA and the USAF, realizing that humans in the space environment would encounter ionizing (particulate) radiations for which the risk factors were unknown, pooled their resources and exposed rhesus monkeys to single "whole body" doses of x-rays, protons (energy range: 10-2300 MeV) or electrons. After the acute study was completed, 301 animals which had received low or intermediate doses between 1964 and 1969 (plus 57 controls) were retained for studies of late radiation sequelae. Thirty-four years later, the Delayed Effects Colony continues to provide data which will improve the quality of radiation risk estimates not only for humans in space but also for individuals exposed on Earth. The objective of the work proposed is to maximize the quality of data produced as the experimental subjects approach the end of their life spans. To that end, NASA was asked to support the surviving monkeys and the program for at least 4 more years. The hypothesis is that the *rhesus macaque* is a model so close to humans that late effects can be extrapolated directly from monkey to human.

The method of approach involves continued care of the subjects and monitoring of all stochastic and deterministic effects that develop. The work to be accomplished includes continuing: 1) semiannual physical examinations; 2) pathological examinations of subjects to measure stochastic effects; and 3) analysis of cataract data from the subjects plus other species (including humans) to extrapolate deterministic effects more effectively to humans. Projects depending on continued NASA support of the subjects include 1) evaluation of genetic damage by measuring persistent chromosome translocations, and 2) continuing measurements of radiogenic cataracts. Expected results include information on late stochastic and deterministic effects, and chromosome

aberration dose response curves, both of which will be relevant and applicable to space radiation risk estimates and to "biodosimetric" analysis of cells from humans exposed to unknown radiation doses.

During FY97, 6 subjects from the so-called "solar flare" (mixed proton energies of 10 and 110 MeV in a ratio of 9:1) were examined post mortem. Three subjects were unirradiated controls and 3 had been exposed to total nominal/surface doses of 3-6 Gy in 1969. All individuals were estimated to be 30-31 years of age at their deaths. All animals showed signs of heart disease, and to some extent, renal and hepatic disease when examined. One out of the three controls and two of the 3 irradiated monkeys which died this year presented intestinal tumors. There are five surviving animals in the "solar flare" cohort at this writing (one control and 4 exposed to total nominal/surface doses of 3 or 6 Gy). Six individuals survive from the other cohorts of monkeys comprising the Delayed Effects Colony for a total of 11 of the original subjects (7% controls and 0.33% treated)(approximate age range of survivors: 31-35 years).

Considering the ages of the surviving individuals, including four younger controls (approximately 25 years of age) we are following, we predict that all the Delayed Effects Colony animals will have died by old age by the year 2001. In the meantime, we have continued to collect and freeze in liquid nitrogen sterile skin and tumor samples (when possible) and non-serile organ and tumor samples in most instances. As the need for primary veterinary care diminishes through the year 2001, we plan to examine more the preserved (frozen) tissues for aging and radiation (carcinogenic) effects *in vitro* (along the lines of earlier published studies using such tissues in the early 1980s), and where necessary, complete histopathological analysis of accumulated tissues and reevaluate fixed normal and tumor tissues.

It is critical to maintain the surviving animals through the ends of their life spans in order to see any tumors that might be developing under the influence of earlier radiation treatments and/or old age. During the past 10 years, especially when one considers the numerous intestinal tumors noted in the monkeys, there have been findings, related to space radiation risk estimates, which had not been expected when the study was first designed. For that reason, NASA is to be commended for its continuing support of the monkeys in the Delayed Effects Colony study, and for its plans to see the project through to the end (of the subjects' life span).

The major goal of this research is to determine radiation risk estimates for humans exposed to ionizing radiations in the space environment. As such, the goal of the project is not to seek new therapeutics but to yield new understanding of basic biological processes. There have been several "spin-offs" of this research which have an impact on humans. The most important of these is the discovery that ionizing radiations appear to increase significantly the incidence and severity of the disease endometriosis in female monkeys. Standard diagnostic radiation doses do not cause this disease, but relatively low doses of environmental radiations can do so in monkeys. Because of the publication of these results in 1991 by Fanton and Golden, other scientists examined female monkeys exposed to the environmental contaminant dioxin, and found that those monkeys also developed excess levels of endometriosis. It is important to emphasize that this result could not have been obtained from standard laboratory animals such as rodents because these animals exhibit a very different type of reproductive cycle from that of nonhuman and human primates. The Endometriosis Association invited Dr. Cox to present the NASA/USAF monkey endometriosis findings at their November 1995 meeting, a recognition that this project has produced data with a direct impact on women, on Earth and/or in space.

The fact that rhesus monkey chromosomes can be treated with the reagents (molecular probes) designed to study aberrations in specific human chromosomes demonstrates the close genetic relationship between humans and macaques. In addition, without developing any new probes, macaque chromosomes can be studied in the same way that human chromosomes are studied in the modern genetics laboratory using Fluorescence *In Situ* Hybridization ("FISH") techniques. Dr. Lucas and his colleagues at Lawrence Livermore National Laboratory, who did the monkey chromosome studies for us, have been funded by the USAF and other agencies to study chromosome translocation phenomena in humans exposed to environmental chemicals such as benzene. The monkey model once again has provided us with data relevant not only to the space environment but to the terrestrial one. The human "FISH" techniques could not have been applied to laboratory rodents at the time we began to use the available molecular probes.

The publication by Di Carlo et al. on Optical Coherence Tomography (OCT) measurements of cataracts in rhesus monkeys is an example of a biomedical technique which could not have been developed utilizing humans. In western countries, when humans develop cataracts, corrective surgery usually is performed before the lens loses full function. Our monkey database on radiogenic cataracts plus the surviving individuals that had or have cataracts, enabled a group of scientists and engineers to cross-correlate two different cataract scoring systems (developed for humans and monkeys) plus the quantitative OCT measurements to give an accurate representation of cataract severity in our irradiated and aging monkeys. This new database will be applicable, in turn, to comparative quantitative measurements on humans suffering from a variety of cataract types, and may serve to aid in diagnoses and prognoses for those human patients. An extension of the OCT work, including extensive histopathological correlations with extant *in vivo* cataract measurements, is being prepared for submission to a peer-reviewed journal.

Since 1986, Dr. Cox has discussed the possibility of gaining access to some of the data on radiogenic cataracts in selected participants in the Adult Health Study (AHS) at the Radiation Effects Research Foundation (RERF) in Hiroshima. Negotiations have been successful, and we started working with the data there, supported by NASA International Programs, during 1996 and 1997. The impact of this part of our project has already begun to be seen. We gave seminars at the RERF in 1993 (with NASA's support and encouragement) and discussed several aspects of our nonhuman primate work with the personnel there. We suggested that the physicians at the RERF examine the eyes of the AHS participants more thoroughly than they have for a number of years based on the late (radiogenic and senile) cataracts we are seeing in the monkey study. If funding for the medical ophthalmological studies is forthcoming, not only will valuable late radiogenic cataract data be obtained for thousands of study participants, but also treatable ophthalmological problems, which can be detected only after pupil dilatation, will become apparent, we hope, before serious visual impairment occurs. This should prove a great relief to that particular population. Some new ophthalmological equipment, developed by a Japanese company, has just been approved for use in humans by the Japanese Ministry of Health and the US Food and Drug Administration (October, 1997). The equipment in question should enable physicians to examine human ocular lenses, and researchers to evaluate nonhuman primate lenses without the need for pupil dilation. This development will be pursued in FY98. We examined medical chart data including drawings and descriptions of lenticular opacifications over (early post-irradiation) time for 67 AHS participants selected for us by the RERF medical and scientific staff. Thus far, the kinetics of apparent radiogenic cataract development are very similar to those seen in our long-lived animal models, but as expected, the cataracts take longer to develop in the human study participants than in the relatively shorter-lived animal models.

Colon cancer and heart disease are problems associated with aging in humans as well as in Rhesus monkeys. It is hoped that the rhesus macaque will be considered as a model for both types of disease and that our data will be applicable to human risk estimates for aging as well as for space radiations. We continue to see cancers of the small intestine in irradiated aging nonhuman primate subjects and cancers of the colon in aging control primate subjects as they succumb to old age. Histopathological data currently are being reevaluated and updated for all cancers seen in the Delayed Effects Colony.

FY97 Publications, Presentations, and Other Accomplishments:

Cox, A.B., Lett, J.T., Lee, A.C., Williams, G.R., and Neriishi, K. "Radiation cataracts: Extrapolation from animal models to humans." (Poster) Annual Meeting of the Association for Radiation Research and the 28th Annual Meeting of the European Society for Radiation Research, Oxford, UK (September 24 - 26, 1997).

Lucas, J.N. "Dose reconstruction for individuals exposed to ionizing radiation using chromosome painting." *Radiat. Res.* (In Press).

Human Enzymatic Repair of Radiation-Induced DNA Breaks

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

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Task Description:

Astronauts receive relatively high exposures of cosmic radiation, putting them at long term risk for radiation-induced cancer. Despite the fact that DNA damage has been shown to be the target for radiation carcinogenesis, the molecular events leading from original exposure to cancer are poorly understood. Consequently, our ability to predict risk from a given radiation exposure is limited. This project is designed to answer some of the basic questions concerning human repair of DNA damage and shed light on some of these basic mechanisms.

We will use DNA strand breaks as a model of a radiation-induced DNA lesion. Recent findings on the exact chemistries of radiation-induced DNA strand breaks have identified the nature of the substrate on which strand-break-repair enzymes must act, and have also revealed the requirement for DNA polymerase in the repair process. This knowledge has widened our understanding of radiation-induced strand breaks from simple biophysical interruptions of the DNA double helix, to specific biochemical lesions that must be modified by multiple enzymatic activities before the DNA can be restored. The human enzymes responsible for 3'-end-group modification (3'-EGMEs) represent the missing link in the strand-break repair process. This proposal seeks to discover the mechanisms of human cellular strand-break repair by directly studying 3'-EGMEs in human cell systems. The proposal concentrates on end-group modification of 3'-phosphoglycolate (3'-PG) to test the hypothesis that DNA strand breaks are of fundamental importance to repair mechanisms for radiation damaged DNA. We plan to characterize human repair enzymes and their mechanisms at the molecular level and determine the effects at the cellular level. State-of-the-art molecular biology approaches will be used to directly probe long-standing radiation biology questions.

The role of DNA strand breaks in mutagenic/carcinogenic outcome. Using the radiomimetic drug, bleomycin, we previously determined the mutagenic potential of DNA strand breaks in the shuttle vector pZ189 in human fibroblasts. The bleomycin conditions used produce strand breaks with 3' PG termini as >95% of the detectable dose-dependent lesions. Breaks with this end group represent 50% of the strand break damage produced by ionizing radiation. We found that such strand breaks are mutagenic lesions.

The type of mutation produced was largely determined by the type of strand break on the plasmid (i.e., single vs. double). Mutagenesis studies with purified DNA forms showed that nicked plasmids (i.e., those containing single strand breaks) predominantly produce base substitutions, the majority of which are multiples, which

presumably originate from error-prone polymerase activity at strand breaks sites. In contrast, repair of linear plasmids (i.e., those containing double-strand breaks) mainly results in deletions at short direct repeat sequences, indicating the involvement of illegitimate recombination. The data characterize the nature of mutations produced by single- and double-strand breaks in human cells, and suggests that deletions at direct repeats may be a "signature" mutation for the processing of double-strand breaks.

This has very important implications for problems regarding the relative biological effects of radiations of different qualities. For example, if mutagenic outcome can be tied to a specific form of DNA damage, biological outcome should be the same regardless of the type of radiation (e.g., HZE vs. x-rays) that produced the lesion. Thus, lesion production becomes the primary predictor of the biological endpoint, and yield can be assessed directly as a form of biological dosimeter.

The mutagenic potential of DNA strand breaks in cells defective in their strand-break repair capacities. Assessment of biologically relevant endpoints in cells defective in specific proteins is a powerful tool to assess the role of those proteins in determining the biological endpoint. In our case, we sought to determine whether cells deficient in proteins, that are known to be responsive to DNA strand breaks, had altered mutagenic potential for strand break mutagenesis.

The fidelity of double-strand-break repair was compared in ataxia telangiectasia (A-T) fibroblasts. The A-T cells are radiosensitive and contain a defect in a single gene (ATM). The predicted gene product of ATM has homology with DNA-dependent protein kinase (DNA-PK). The A-T cells showed a 2 to 3-fold increase in mutagenesis compared to the normal fibroblast cell line, WI-38. These results suggest that loss of repair fidelity may contribute to some of the phenotypes observed in these cell lines, such as their cellular radiosensitivity, and perhaps the cancer proneness seen in A-T. Cellular A-T phenotypes, such as radiosensitivity and genomic instability, suggest that a deficiency in the repair of DNA double-strand breaks (DSBs) may be the primary defect; however, overall levels of DSB rejoining appear normal. We used the shuttle vector, pZ189, containing an oxidatively-induced DSB, to compare the fidelity of DSB rejoining in A-T and normal fibroblasts. Mutation frequencies were not only higher, but also the mutational spectrum was different. The deletions in plasmids recovered from normal cells were always between short direct repeat sequences, implicating illegitimate recombination in DSB rejoining. Deletions in A-T did not occur at direct repeats, suggesting a defect in illegitimate recombination. These findings suggest that the A-T gene product may either directly participate in illegitimate recombination or modulate the pathway. Regardless, this defect is likely to be important to a mechanistic understanding of DNA double-strand break repair.

The majority of known human carcinogens have been shown to be potent mutagens, and mutagenesis is thought to be the principle mechanism by which cancer is initiated. Ionizing radiation is a known mutagen, and mutation of key target genes within irradiated cells is probably the initial (irreversible) event which starts a cell on the pathway to tumorigenesis. Cellular DNA repair systems can both mitigate and potentiate the mutagenic consequences of radiation-induced DNA damage through a variety of "error-free" and "error-prone" repair pathways. Understanding these pathways, and the environmental factors that influence them, is probably key to understanding the mechanisms of mutagenesis and cancer induction in man, as well as the cancer risk associated with radiation exposure.

FY97 Publications, Presentations, and Other Accomplishments:

Dar, M.E., Winters, T.A., and Jorgensen, T.J. "Identification of defective illegitimate recombinational repair of oxidatively-induced DNA double-strand breaks in ataxia-telangiectasia cells." *Mutation Res. DNA Repair*, 384, 169-179 (1997).

Dar, M.E., Winters, T.A., and Jorgensen T.J. "Identification of defective illegitimate recombinational repair of oxidatively-induced DNA double-strand breaks in ataxia-telangiectasia cells." (Abstract) Annual Meeting of the Radiation Research Society, Providence RI (1997).

Mutations in Human Lymphoid Cells

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Post-Doctoral Associates: 2

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The goals of this proposal are to determine the susceptibility of human cells to mutagenesis following exposures to charged particle radiations found in space. We are studying the heritable alterations produced in human lymphoblasts following exposures to protons or iron ions. Protons are the dominant component of the space radiation environment. While iron ions are much less abundant, they are thought to be much more damaging to cells and tissues due to their energy deposition characteristics. We will determine the effects of certain biological variables (e.g., gene copy number, genetic linkage, and the expression of genes that may regulate the stability of the human genome) on cellular susceptibility to mutagenesis and cytotoxicity following exposure to low doses of protons or iron ions.

These studies are being performed with syngeneic human B-lymphoblastoid cell lines that are respectively expressing either only normal p53 or only mutated p53. The p53 tumor suppressor gene is mutated in a wide variety of human tumors. In addition, mutations in p53 are often associated with an increased resistance to the toxic effects of ionizing radiations. We have shown that cells expressing mutant p53 are indeed more resistant to the toxic effects of either protons or densely ionizing iron ions. In addition, cells expressing mutant p53 are more easily mutated by either protons or iron ions than cells that express normal p53 protein. The increase in mutation susceptibility is much more pronounced for the autosomal thymidine kinase (tk) locus than for the X-linked hypoxanthine phosphoribosyltransferase (hprt) locus.

Molecular analysis of mutations will provide information on the types of heritable DNA structural alterations that result from the traversal of human cells by low fluence exposures to Fe ions and to moderate doses of

protons. The characterization of mutant spectra is ongoing in both the p53 mutant cell line and in the cells that express normal p53.

In FY 97, we focused on the clonal isolation of a series of mutant cell lines derived from two syngeneic human B-lymphoblastoid cell lines, TK6 and WTK1. The TK6 cell line expresses normal p53 protein, while the WTK1 cell line has homozygous mutant p53. We exposed each of these cell lines to an average of 3 particles/cell of 1 GeV 56Fe ions at the Alternating Gradient Synchrotron and used biochemical screening techniques to isolate mutants deficient in either hprt or in tk. Based on our analysis of the surviving fractions, we determine that most of the mutants that we isolated arose as a result of a single Fe ion traversing a given cell at risk. We isolated 85 hprt-deficient mutants, 36 derived from TK6 cells and 49 derived from WTK1 cells. We also isolated 198 tk-deficient mutants, 99 derived from TK6 cells and 99 derived from WTK1 cells.

Earlier, we had shown that the difference in susceptibility to Fe ion-induced mutation was not strongly impacted by the functional status of the p53 protein. Only a two-fold enhancement in mutation susceptibility was seen in the WTK1 cells with mutant p53. This year, we used a PCR-based approach to characterize molecular changes at the hprt locus. We determined that deletion mutations were the most common (58% of TK6 cells demonstrated loss of all or part of the hprt gene, while 67% of WTK1 cells demonstrated loss of all or part of the hprt gene). A comparison of the molecular alterations seen among the TK6 and WTK1 derived mutants showed that the mutant spectra did not differ (χ^2 , 2 df = 1.21). This suggests that the functions modulated by normal p53 (e.g., predisposition to apoptosis, or modulation of double strand break repair capacity) did not markedly impact the nature of the mutations tolerated following densely ionizing radiation exposure in these human lymphoid cell lines derived from the same original donor.

In contrast to what we had observed for the hprt locus, we had shown that WTK1 cells were mutated 25-45X more frequently at the tk locus than were TK6 cells. This year we demonstrated that the predominant mechanism through which mutants arose at the tk locus was via loss of heterozygosity (LOH). In the TK6 cells, 40/47 mutants that appeared at early times post-irradiation arose by LOH, while among those that appear at later times, post-exposure 48/49 mutations arose by LOH. A similar pattern was observed among the WTK1-derived tk-deficient mutants. In the WTK1 cells, 45/49 early arising mutants demonstrate LOH, while 49/50 late-arising mutants demonstrate LOH. Thus, low fluence exposures to Fe ions are associated with a high proportion of autosomal mutations that arise through LOH. At the present level of analysis, we do not see major differences in mutant spectra between the p53 mutant WTK1 cells and the TK6 cells with normal p53 protein.

We developed a methodology to determine gene copy number at the thymidine kinase locus in mutants demonstrating LOH. This analysis will allow us to distinguish whether LOH has occurred through simple deletion of the previously active tk allele or whether it has arisen by loss of the active allele and reduplication of the silent allele. These studies are ongoing in the laboratory, as are long range mapping studies using a series of microsatellite markers along the length of chromosome 17q. The mapping studies will allow us to determine the length of the LOH tracts that arise following exposure to a very low fluence of densely ionizing radiations. Together, these approaches will allow us to determine the mechanisms of mutagenesis at a standard autosomal locus, and will allow us to detail the influence of p53 function on the types of mutations arising after low fluence exposure to Fe ions.

Also in FY97 we carried out a preliminary study that suggests that an exposure to 3 particles/cell of Fe ions can induce apoptosis efficiently in TK6 cells with normal p53 protein. In contrast, we did not detect apoptosis in the WTK1 cells that express only mutant p53.

Although densely ionizing radiations are of importance for long duration manned spaceflight outside the magnetosphere, sparsely ionizing protons are of great importance in both near-Earth orbit and interplanetary flight due to their abundance.

Our studies are directed to understanding the importance of a variety of genetic factors in the susceptibility to the accumulation of heritable alterations in somatic cells. These studies are directly relevant to the types of

alterations that occur in human cancer. We have shown that different genes in the human genome have different susceptibilities to mutation induction following exposure to clastogens—in this case, different types of ionizing radiations. The magnitude of susceptibility is directly associated with the position of the gene of interest relative to flanking essential genes and to gene copy number. A wide variety of clastogenic chemicals are found in nature in addition to physical clastogens, such as x-rays and other forms of ionizing radiation. Our studies are also important in understanding basic biological processes associated with radiation exposure. Our data demonstrate that large deletion mutations are readily accumulated following low dose exposures to ionizing radiation and that such mutations can be stably maintained if they occur in non-essential parts of the genome. In addition, our preliminary studies suggest that the p53 gene, which is mutated in a large number of human tumors, is an important determinant of the frequency with which additional mutations are accumulated within cells at risk. Cells with a pre-existing mutation in p53 are more likely to accumulate additional genetic changes upon exposure to a mutagen such as ionizing radiation than are cells that have normal p53. As the p53 gene regulates diverse cellular processes including transcription, DNA repair, and apoptosis, our results are pertinent to the progression of pre-cancerous lesions in humans following repeated exposure to the wide variety of mutagens we encounter in everyday life on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Kronenberg, A. "Biological consequences of exposure to the space radiation environment." Lecturer in Human Biology 107, Astrobiology, Stanford University (Fall, 1996).

Kronenberg, A., Cherbonnel-Lasserre, C., Gauny, S., Liu, W., and Wiese, C. "Suppression of apoptosis by bcl-2, bcl-XL, or mutant p53 is associated with increased mutagenesis in human cells exposed to clastogenic agents." Cold Spring Harbor Symposium on Programmed Cell Death, Cold Spring Harbor, NY (1997).

Kronenberg, A., Gauny, S., Cherbonnel-Lasserre, C., Liu, W., and Wiese, C. "Effect of p53 status on cell killing and mutation induction following low fluence exposure to 1090 MeV/amu Fe ions: Progress report on analyses of intragenic mutation spectra at the hprt and tk loci." 8th Annual Investigator's Workshop in Space Radiation Research, Upton, NY (1997).

AGS Beam Time (HZE Particles)

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-45-17-13

Solicitation: not available

Initial Funding Date: 1996

Expiration: 2002

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$836,242

Task Description:

Manned space exploration in the 21st century holds exciting prospects for the advancement of science and the expansion of the human experience. Plans include the International Space Station, an outpost on the Moon, exploration of near asteroids, and a piloted mission to Mars. However, for space exploration to go on, human crewmembers must be protected against the harsh environment of space, in particular, against the hazards of ionizing radiation. The radiation environment in space consists of high-energy protons and high-energy heavy ions (HZE). Knowledge of the biological effects of HZE ions has important implications for human exploration of space. The principal source of HZE ions in nature is galactic cosmic rays (GCR), which consist mostly of protons, with small components of helium and heavier nuclei, electrons, and positrons. Although the GCR will be attenuated and fragmented by electromagnetic and nuclear interactions in shielding material, crewmembers will still be exposed to significant radiation from both primary and secondary nuclei.

Research supported by NASA on the radiobiological effects of high-energy heavy ions had been carried out for several years at the Lawrence Berkeley National Laboratory BEVALAC in California. With the closing of the BEVALAC, the Brookhaven National Laboratory (BNL) Alternating Gradient Synchrotron (AGS) is the only accelerator in the United States capable of providing heavy ion beams at energies of interest for space radiobiology.

The activities under this task include study of heavy ion fragmentation relative to space radiobiology, and the genetic and epigenetic consequences of heavy ions (^{56}Fe) in a hierarchy of biological systems using the AGS at BNL. The biological effects of this densely ionizing radiation were studied in a number of genetic and cellular endpoints having a genetic component, constituting a broad range of quantifiable endpoints from the molecular to the integrated tissue level. The biological models used range from a simple system in which damage is studied in isolated DNA to complex endpoints in which integrated tissue responses may influence the observed effects. The biological experiments were supported by a physics component designed to provide basic dosimetric information as well as the detailed characterization of the radiation field essential for the accurate interpretation of the biological data.

There are, in addition, the following objectives related to the establishing the capability and protocols for performing radiobiology experiments at the AGS: (a) Measure beam characteristics: intensity, energy spectra,

purity, spill structure; maximum and minimum reliable doses rate; and maximum and minimum reliable beam spot sizes; (b) Establish and test instrumentation for beam monitoring and control; (c) Establish consistent dosimetry and beam characterization; and (d) Develop biological system handling procedures specific to the AGS and BNL.

During the Fall of 1996, a second group of radiobiological and physics experiments were performed using the Brookhaven National Laboratory Alternating Gradient Synchrotron that it is able to generate high energy ^{56}Fe ion beams (Experiment 898, BNL-2). The run's primary goals were (1) the continuation of the use of the 1 GeV/nucleon iron beam for biology and physics experiments employing a tested dosimetry system, and an improved logistic support organization, and (2) the establishment of the capability and protocols for experiments using 0.6 GeV/nucleon iron beams.

A total of 18 groups participated in the BNL-2 run; 12 groups were returnees from 1995's BNL-1, and 6 groups were new participants. These groups represented 16 institutions from United States, and totaled 63 scientists. Their experiments were dedicated to the study of the physics characteristics and the biological effects of ^{56}Fe ion beams on detectors, and a hierarchy of biological systems ranging from isolated DNA, to cells, tissues and intact organisms.

A total of 1400 biological samples were irradiated at the AGS A-3 beam line, employing 85 hours of beam time. In addition, 44 hours were used for physics experiments, and a total of 22 hours were necessary for beam characterization, dosimetry, and calibration. During the BNL-2 run, AGS provided iron beams with two energies: 1 GeV/n (1.06 GeV/n on target, LET: 148 keV/ μm) for biology and physics, and as a test mode, 0.6 GeV/n (0.575 GeV/n on target, LET: 175 keV/ μm) mainly for physics and for a limited group of biology experiments. The dose/rates used were as low as 1cGy/min and as high as 15 Gy/min for 1 GeV/n, and from 3-5 cGy/min up to 2 Gy/min for 0.6 GeV/n iron beams. The spill rate employed was 30 spills/min with a length of 500 msec. The intensities (particles/ cm^2 /spill on target) used during the run were 1.4×10^5 - 9×10^6 for 0.6 GeV/n, and 1.5×10^5 - 7.5×10^7 for 1 GeV/n. A 7.5 cm diameter beam spot was employed for the exposures. More than 90% of the experiments and beam time used 1 GeV/n Fe. In general, the users were able to complete their experiments. In addition, AGS was able to deliver 0.6 GeV/n iron beams, demonstrating that this tune modality can be included in future run beam manifests.

Several changes have been introduced for BNL-2's logistic support: a new medical department liaison, a better coordination of laboratory arrangements, the use of a general questionnaire to support logistics requirements, streamlined safety training, and an improved run schedule organization and implementation. All these changes were reflected in better utilization of the available beam time considering the increase in number of users (53.6%) and samples exposed (57%). Finally, the growing maturity of the facilities, personnel supporting the run, and users, was reflected in an increase beam availability, more flexible problem solving strategies and optimization of the beam time allocated to the users.

List of experimental groups

B1a PI: M. H. Barcellos-Hoff, Lawrence Berkeley National Laboratory
Title: Epithelial Transformation and Carcinogenesis

B1b PI: P. Cooper, Lawrence Berkeley National Laboratory
Title: DNA Damage and Repair in Mammalian Cells

B1c PI: A. Kronenberg, Lawrence Berkeley National Laboratory
Title: Mutagenesis in Human and Rodent Cells

B1d PI: G. Nelson, NASA, Jet Propulsion Laboratory
Title: Studies of Mutations and Chromosomal Aberrations in the Nematode *C. elegans*.

- B1e PI: J. Miller, Lawrence Berkeley National Laboratory
Title: Small Angle Fragment Fluence Spectra at Depth in Cells and Tissues
- B1f PI: M. Vazquez, Brookhaven National Laboratory
Title: The Effects of High Energy Heavy Ions on Neural Plasticity
- B2 PI: D. Chen, Los Alamos National Laboratory
Title: Effect of Charged Particle Track Structure on Radiation Mutagenesis
- B3 PI: T C. Yang, NASA Johnson Space Center
Title: Quantitative Studies on the Oncogenic and Cytogenetic Effects of Energetic Iron Particles in Mammalian Cells.
- B7 PI: B. Rabin, University of Maryland Baltimore County
Title: Effects of Exposure to Heavy Particles
- B8 PI: T. Jorgensen, Georgetown University
Title: DNA Strand Breaks Produced in Mammalian Cells by Heavy Ion Irradiation.
- B9 PI: B. Sutherland, Brookhaven National Laboratory
Title: DNA Damage and Restoration in Mammalian Cells and Tissues
- B11 PI: L. Lutze-Mann, University of California San Francisco
Title: Molecular Analysis of HZE Damage in Transgenic Mice
- B12 PI: T. Hei, Columbia University
Title: Cytogenetic and Neoplastic Transforming Effects of Heavy Ions in Mammalian Cells
- B13 PI: E. Balcer-Kubiczek, University of Maryland at Baltimore
Title: Molecular Damage by 1 GeV/amu Fe Ions.
- B14 PI: N. Meeting, Pacific Northwest National Laboratory
Title: The Effect of Heavy Ion Exposure on a Mechanism of Cell Cycle Regulation.
- B15 PI: C. Waldren, Colorado State University
Title: HZE Radiation Genotoxicity in Cultured Mammalian Cells.
- B16 PI: J. Lett, Colorado State University
Title: Cell Cycle Responses of DNA Damage and Repair in L5178Y S/S Murine Leukemic Lymphoblasts Exposed to ⁵⁶Fe Ions.
- B17 PI: A. Lindgren, Bemidji State University
Title: RBE of ⁵⁶Fe on Rodent Lens Epithelia.

This research is related to the effects on humans of space radiation with the ultimate goal of providing a firm scientific basis for space radiation protection. These experiments address the particular problem of high-energy heavy ion radiation exposures during future long-term deep space flights. The principal objective is to improve our understanding of the biological effects of low fluences of densely ionizing charged particles on living cells and tissues.

In addition, these studies have the potential for enhancing and extending our knowledge of the structure and reparability on genetic material, and understanding the link between ionizing radiation and biological effects such as mutagenesis, cancer, and aging. Although the radiation fields encountered in space are both quantitatively and

qualitatively different from those on Earth, comprehension of their mechanisms of action will increase understanding of basic mechanisms of action of ionizing radiation, and the use of high energy charged particles in radiotherapy.

FY97 Publications, Presentations, and Other Accomplishments:

Gatley, S.J., Vazquez, M.E., Pyatt, B., Lindgren, A., and Volkow, N. "Measurement of dopamine receptors and transporters in rats after irradiation with 1 GeV/amu ⁵⁶Fe beam." NASA, 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY (April 29 - May 3, 1997).

Lowenstein, D., Pile, P., and Lazarus, D. "AGS operations." NASA, 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton NY (April 29 - May 3, 1997).

Sutherland, B.M., Bennet, P.V., and Sutherland, J. "DNA double strand break quantitation in human cells irradiated with cGy doses of Fe (1 GeV/amu) ions." NASA, 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY (April 29 - May 3, 1997).

Sutherland, B.M., Bennet, P.V., and Sutherland, J.C. "Quantitation of double strand breaks at low frequencies in human DNA irradiated in solution and in cells." Forty-fifth Annual Meeting of the Radiation Research Society, Providence, RI, pp:198, P20-408 (1997).

Vazquez, M.E. "The effects of 1 GeV/amu ⁵⁶Fe Ions on neurite outgrowth in retinal explants." Forty-fifth Annual Meeting of the Radiation Research Society, Providence, RI, pp:220, P24-493 (1997).

Vazquez, M.E. and Heilbronn, H. "BNL-2 run summary." NASA, 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY (April 29 - May 3, 1997).

Vazquez, M.E. and Kirk, E. "*In Vitro* Neurotoxicity of 1 GeV/amu ⁵⁶Fe Ions." NASA, 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY (April 29 - May 3, 1997).

Guidance on Space Radiation Risks

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-45-17-10

Solicitation: not available

Initial Funding Date: 1995

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$30,000

Task Description:

The National Council on Radiation Protection and Measurements (NCRP) is to conduct a study on the use of fluence as the basis of a radiation protection system for astronauts. NCRP Scientific Committee 88, chaired by Dr. Stanley Curtis, has been established to do this review. They are to review the absorbed dose method, the microdosimetry and the fluence method of evaluating exposure of astronauts to cosmic radiation found in space. The NCRP is to also review methods of extrapolating radiation risk information from animal studies to humans. NCRP Scientific Committee 1-4, chaired by Dr. David Hoel, has been established to do this review. The NCRP is also to advise on research needed to be conducted in order to make radiation protection recommendations for humans involved in deep space missions such as colonizing the moon or a mission to Mars. This work is being done by NCRP Scientific Committee 1-7, chaired by Dr. Lawrence Townsend. All three of these reviews are to be published as NCRP reports.

The NCRP is in the last year of two projects and the next to last year of a third project for NASA. NCRP Scientific Committee 88 is in the last year of a report writing effort dealing with the evaluation of fluence as a dosimetry system for astronauts and NCRP Scientific Committee 1-4 on Extrapolation of Radiation Risks from Animals to Humans is in the last year of report writing effort. NCRP Scientific Committee 1-7 on Research Needs for Making Radiation Protection Recommendations for Humans Involved in Deep Space Activities is in its next to last year in its report writing activity. All three committees will draft NCRP reports, which when approved by the Council membership, will be published as NCRP reports. There are no obvious problems known that might hamper publication of these reports.

Completion of the work being performed by NCRP Scientific Committee 1-4, 1-7, and 88 should significantly improve our estimates of the risk to humans from high linear-energy-transfer (LET) radiations such as those found in space as well as low-LET radiation risks that are currently based solely on the results of studies of the survivors of Hiroshima and Nagasaki. Completion of this work will also lead to improved radiation protection practices in space activities as well as for radiation protection programs on Earth.

The Effect of Single Particle Traversals on a Mechanism of Cell-Cycle Regulation

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-45-17-18

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 193,631

Task Description:

We propose to test the hypothesis that passage of high energy heavy ions through the cell nucleus results in altered subcellular localization of certain cell-cycle regulatory proteins. In addition, we have expanded the scope of this project to include assaying for *in situ* endonuclease activity, a DNA repair activity that is probably linked to initiation of cell-cycle delay. We continue this work with major emphasis on DNA repair proteins. The carcinogenic effects of high-LET radiation, such as the heavy particle component of galactic cosmic rays, are a major concern in long-duration space missions, and it is clear that perturbations in normal cell growth regulatory systems, and more importantly in DNA repair systems, make up one or more components of the multistep process of carcinogenesis.

As was stated last year, the project began as a study of cell-cycle regulatory responses to high-LET particle traversal, but within the first six months of work, the research took on an entirely new direction when it was realized that we could visualize heavy particle traversals by assaying for *in situ* endonuclease activity. At that time, the PI suggested that this was a DNA repair activity that was probably linked to initiation of cell-cycle delay. We continued this work with major emphasis on DNA repair proteins, using human cells exposed to gamma-rays, alpha particles, and high energy iron ions. The early-responding DNA repair proteins, Ku-86 and MLH-1, were studied in gamma ray irradiated human neo-natal foreskin fibroblasts using immunocytochemistry. After irradiation, the cells were incubated for 0.25, 0.5, 1, 2, 4, or 8 hours before fixation in methanol and immunostaining. After a dose of 50 cGy, this time course shows a steep up-regulation of Ku-86 in cell nuclei 7.7x over unirradiated cells by 15 minutes, 16.8x in 1 hour, then a more gradual climb to a high of 25.4x at 4 hours. The dose response curve for doses of 10, 50, 100, 200, 400, and 800 cGy after one hour of incubation show a very interesting pattern of up-regulation. If the two lowest doses are not considered, the dose response curve would show a nearly linear increase in labeling intensity that was proportional to dose and rose to 13.8x over background. The low doses add quite an interesting higher bend to the curve: at ten cGy, the increase is 18.4-fold over background, and at 50 cGy, the increase is 15.5-fold higher than background. We see a similar dose response for MLH-1 in the first, but not the second, of two experiments with this second protein. The pattern of Ku-86 labeling across the nucleus is fairly homogeneous, with several slightly denser areas that may be up to 4-fold heavier than the surrounding nuclear area. We have made some progress in immunostaining for these proteins in cells exposed to 1 GeV/amu iron ions. We see labeling in very discrete volumes within the cell nucleus, but these nuclei are also lightly stained throughout. Even more striking is the induction of Ku-86

in alpha-particle-irradiated HeLa cells, incubated for 2 hours before fixation and staining. In addition to an up-regulation in excess of 20-fold, the staining is clearly heaviest in from three to six nuclear foci.

These data are consistent with the following conclusions: 1) many fold higher levels of Ku-86 are available for immunolabeling as early as 15 minutes after ionizing radiation exposure; 2) particle irradiation concentrates DNA damage in discrete nuclear volumes to which repair proteins are drawn; and 3) cell morphology is important for this visualization technique, so additional experiments must be done using cells of a more normal genotype than HeLa, but with a morphology that is like HeLa in that it is more rounded than a flat skin fibroblast. To this end we have begun to use normal human epithelial cells of several types. For the BNL3 experiments of October 1997, we will use HMEC human mammary epithelial cells (Clonetics, Inc.), and will be analyzing these experiments in the coming months.

The carcinogenic effects of high-LET radiation such as the heavy particle component of galactic cosmic rays are a major concern in long-duration space missions. Estimation of cancer risk from exposure to this environment would benefit from greater knowledge of the cellular effects of individual particles. But there is an even greater need for this type of data in the estimation of risk from inhaled radon on the planet Earth. That is because the very act of inhalation serves to guide a ubiquitous, airborne, high-LET radiation source into contact with a sensitive population of body cells. The Earth's crust releases differing amounts of radon into the atmosphere to be breathed, hence there is an advantage in knowing what the risks are so that informed decisions can be made on the placement of dwellings and workplaces for human populations.

The mechanisms of carcinogenesis in general are still not all understood, but it is clear that perturbations in normal cell growth regulatory systems or DNA repair systems make up one or more components of the multi-step process. This work will help answer basic questions of cell-cycle regulation, currently under discussion, relating to mechanisms of checkpoint control at various points of transition in the cell cycle, and may ultimately help resolve the present debate on how the epigenetic mechanism of altered subcellular localization might contribute to the process of carcinogenesis.

FY97 Publications, Presentations, and Other Accomplishments:

Braby, L.A., Brooks, A.L., and Metting, N.F. "Cellular effects of individual high linear energy transfer particles and implications for tissue response at low doses." *Radiat. Res.*, 148, S108-114 (1997).

Ford, J.R., Metting, N.F., Marsden, S.J., Stevens, D.L., Townsend, K.M.S., and Goodhead, D.T. "Visualization of damage generated along alpha particle tracks in irradiated rat tracheal epithelial cells" in "Microdosimetry: An Interdisciplinary Approach." Edited by: Goodhead, D.T., O'Neill, P., and Menzel, H.G. Royal Society of Chemistry, Cambridge, UK, pp 335-338 (1997).

Metting, N.F. "Early cellular responses to 1 GeV/amu iron ion exposure." Presented at the 8th Annual Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, Long Island, NY (April 29 - May 3, 1997).

Metting, N.F. "Early cellular responses to high LET radiation." Seminar for the WSU Genetics and Cell Biology Club, Washington State University, Pullman, WA (March 9, 1997).

Metting, N.F. "Early cellular responses to radiation exposure: Signaling for cell-cycle arrest and DNA repair." Seminar given at the Department of Nuclear Engineering, Texas A&M University, College Station, TX (March 17, 1997).

Metting, N.F. and Braby, L.A. "Visualization of charged particle traversals in cells" in "Microdosimetry: An Interdisciplinary Approach." Edited by: Goodhead, D.T., O'Neill, P., and Menzel, H.G. Royal Society of Chemistry, Cambridge, UK, pp 331-334 (1997).

Metting, N.F., Braby, L.A., and Randers-Pehrson, G. "Visualization of early cellular responses in microbeam-irradiated human cells." Presented at the 3rd International Microbeam Workshop: Microbeam Probes of Cellular Radiation Response, Columbia University, New York, NY (May 8 - 9, 1997).

Experimental Study of Nuclear Interactions Relevant to High Energy Heavy Ion Transport

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Funding:

UPN/Project Identification: 199-45-16-12

Solicitation: not available

Initial Funding Date: 1994

Expiration: 1996

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 175,000

Task Description:

Humans spending extended periods of time outside the Earth's atmosphere and magnetic field or at very high altitudes are exposed to types and doses of radiation not typically encountered at the Earth's surface. The radiation exposure will depend upon the particular mission scenario, such as a space station, interplanetary spacecraft, lunar and planetary habitats, and very high flying aircraft. Assessment and mitigation of the attendant radiation risks requires accurate knowledge of the possible radiation environments and how they are modified by passage through shielding material and human tissue, and of the biological effects of radiation. This project focuses on one particular component of space radiation, the heavy (heavier than hydrogen) nuclei present in the galactic cosmic rays. Its principal aims are to make ground-based measurements (at particle accelerators) of the fragmentation of heavy ion radiation in matter, particularly cells and tissue, to apply this information to the interpretation of measurements of the biological effects of heavy ion radiation, to compare the measurements with the predictions of models of the physical and biological effects of heavy ions, to provide physics support to radiobiologists doing experiments at particle accelerators, and to assist in the training of students and scientists new to the field of accelerator-based radiobiology. Fragmentation measurements are made by placing particle detectors in the path of beams of accelerated heavy ions which pass through biological samples, tissue-equivalent targets such as water or polyethylene, or shielding material such as aluminum. Radiation fields measured behind biological samples provide radiobiologists with a description of the radiation incident on their samples. This information can be important, as some degree of beam fragmentation is unavoidable in an accelerator experiment. Data on fragmentation in tissue and shielding are useful as a direct measure of the effects of the self-shielding of the human body and as input to and benchmarks of models of radiation transport. These models are an essential part of the solution to the space radiation problem, as it is impractical to empirically test the physical and biological effects of every possible combination of radiation environment and shielding material and thickness for every biological endpoint. The activities under this task include experiments at the Alternating Gradient Synchrotron (AGS) at Brookhaven National Laboratory (BNL) Heavy Ion Medical Accelerator (HIMAC) at the National Institute of Radiological Sciences (NIRS), Chiba, Japan, and the 88" Cyclotron at Lawrence Berkeley Laboratory. Measurements at the Loma Linda University Proton Therapy Facility are in the planning stages. These experiments consist of both direct measurements of heavy ion fragmentation relative to space radiobiology and measurements, such as beam characterization, in support of biologists and theoretical physicists working on aspects of the space radiation problem. The data taken during these experiments are analyzed and presented in reports, at conferences, and in peer-reviewed scientific journals. We also collaborate

with theoretical physicists, radiobiologists and biophysicists in areas of mutual interest, and in particular where physics expertise can be brought to bear on problems in space radiation biology.

A series of measurements was made at the BNL AGS. *Fluences* (particle spectra behind thick targets) were measured for a variety of elemental, tissue equivalent and candidate shielding materials. *Cross sections* (particle spectra behind thin targets) were measured behind elemental targets. Data analysis is in progress, and preliminary data have been provided to colleagues at NASA Langley Research Center for comparisons to charged particle transport model calculations. During the next year, additional thin targets will be run, chosen based on the cross sections most needed to constrain the transport codes. Additional thick targets will be run, both to test the transport codes and to study the radiation shielding and transport properties of complex realistic shielding targets provided by NASA-LaRC and NASA-JSC.

During the same accelerator running period, physics support was provided for NASA-sponsored investigators performing radiobiology experiments at the AGS. This included dosimetric and beam characterization measurements. A detailed study was made of the characteristics of the AGS 600 and 1000 MeV/nucleon iron beams relevant to radiobiological experiments. This is available as LBNL Report No. 39267, and will also be published in the journal *Radiation Research*. We also provided physics support for NASA-sponsored radiobiology at the LBL 88" cyclotron. During the next year, we will continue to provide physics support for radiobiology at the cyclotron and the AGS; the principal goals, in addition to this function, are to establish the capability to deliver lighter beams, such as silicon, for radiobiology.

At the AGS, we collaborated with physicists from Colorado State University, supported under the LBL-CSU NSCORT, in a study of TEPC response to high energy heavy ions. This has led to one NSCORT Ph.D. dissertation and a paper which has been submitted for publication. We plan to continue these studies in the coming year, focussing on obtaining higher statistics, and training two new NSCORT graduate students.

The first NASA-sponsored physics measurements were made at the NIRS HIMAC. These measurements were made with lighter ions and lower energies than available at the AGS, and thus complemented the AGS data. Preliminary results of these experiments were presented at the NASA Space Radiation Health Program Investigators' Meeting (May 1997) and the 12th IAA Man In Space Symposium (June 1997). Of particular interest are data on multiple light charged particle production both on and off the beam axis. In the coming year, we will return to HIMAC, and extend these measurements to other ions and targets, to study projectile mass and energy, target mass, and angular-dependence of light fragment production.

At BNL and HIMAC, intercalibrations were performed between a ground-based solid state particle telescope and two international flight radiation monitoring instruments: "Liulin" (STIL-BAS, Sofia, Bulgaria) which was used on the Mir space station and RMRD III (Waseda University, Tokyo, Japan) which will be flown on the Space Shuttle. We plan to extend these measurements to next generation flight instruments.

This research supports radiobiological studies of the effects of high energy heavy charged particles on biological systems. These studies have the potential for improving and extending our understanding of the structure and repairability of genetic material, as well as the link between ionizing radiation and biological effects such as cancer. The radiation fields in space are both quantitatively and qualitatively different from those on Earth; however there are also significant areas of overlap, including the fundamental mechanisms of action of ionizing radiation and the use of high energy charged particles, such as are found in the galactic cosmic radiation, in radiotherapy.

FY97 Publications, Presentations, and Other Accomplishments:

Dachev, T., Semkova, J., Petrov, V., Redko, V., Bengin, V., Kostereva, T., Miller, J., Heilbronn, L., and Zeitlin, C. "Analysis of the pre-flight and post-flight calibration procedures performed on the Liulin Space Radiation Dosimeter." 12th Man in Space Symposium, Washington, DC (June 8 - 13, 1997).

Miller, J., Fukumura, A., Heilbronn, L., Murakami, T., and Zeitlin, C. "Fragmentation of 275 MeV/u ^{12}C in C, CH₂, Al, Cu and Pb." Seventh Annual NASA Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory (April 29 - May 3, 1997).

Miller, J., Zeitlin, C., Heilbronn, L., Borak, T., Carter, T.R., Frankel, K.A., Rademacher, S.E., and Stronach, C.E. "Ground-based simulations of cosmic ray heavy ion interactions in spacecraft and planetary habitat shielding materials." 12th Man in Space Symposium, Washington, DC (June 8 - 13, 1997).

Schimmerling, W., Ainsworth, E.J., Heilbronn, L., Miller, J., Yang, T.C., and Zeitlin, C.J. "The role of projectile fragmentation in heavy ion radiobiology." Proceedings of the Heavy Ion Symposium, Dubna, Russia (January, 1997).

Wilson, J.W., Cucinotta, F.A., and Miller, J. "Radiation shielding issues in highly Inclined low Earth orbits." Proceedings of the International Conference on Environmental Systems, Monterey, CA (July, 1997).

Zeitlin, C., Heilbronn, L., and Miller, J. "Detailed characterization of the 1087 MeV/nucleon ^{56}Fe beam used for radiobiology at the AGS." Lawrence Berkeley National Laboratory Report No. LBNL-39267 (June, 1997).

Zeitlin, C., Heilbronn, L., Miller, J., Rademacher, S.E., Borak, T., Carter, T.R., Frankel, K.A., Schimmerling, W., and Stronach, C.E. "Heavy fragment production cross sections from 1.05 GeV/nucleon ^{56}Fe in C, Al, Cu, Pb and CH₂ targets." Phys. Rev., C 56, 388 (1997).

3D ORAM Dosimeter for Space Radiation Environments

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Funding:

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Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$82,406

Joint Agency Participation: DoE

Task Description:

Our objectives are to develop a crew dosimeter for heavy-charged-particle (HZE) monitoring, applicable to the U.S. space flight program. The dosimeter will enable personnel dosimetry in space radiation environments, providing radiation protection to humans in space. The use of radiation-induced changes in three-dimensional optical random access memories (3-D ORAM) provides the basis for the approach. ORAM is a small cube (a few mm³) composed of transparent polymer doped with a light-sensitive chemical. Two intersecting laser beams are used to write and read binary information (bits) on ORAM which functions as a HZE detector. This dosimeter will be capable of determining both the energy and the type of the HZE particle, and will be orders of magnitude more sensitive and accurate than existing methods.

Initially, theoretical studies were performed to determine the effects of HZE particles on 3-D ORAM and provide direction for an experimental approach. Experimental data was then obtained using thin films of a 3-D ORAM material irradiated with heavy charged particles. The effects of radiation on the films were evaluated using a laser scanning confocal microscope. Permanent changes corresponding to particle energy and dose are observed. This task has consequently been redirected to include identification of the chemical species created and the chemical process by which this permanent change occurs. Further, the direction of this task now includes additional experimental methods, such as near field scanning optical microscopy techniques, for particle identification and to further investigate the effects of HZE particles on 3-D ORAM.

During the third year of the project, we continued experimental research to measure the effects of radiation on 3-D ORAM. Thin films of spirobenzopyran doped poly(methyl methacrylate) were produced on site and irradiated using the tandem accelerator facility at the Naval Surface Warfare Center in White Oak, Maryland. Particle beams of protons (0.6, 1.0, 1.5, 2.0, and 2.5 MeV), alphas (2.0 and 5.0 MeV), and carbon-12 (6.5 MeV) with fluence ranging from 10¹⁰ to 10¹⁴ particles/cm² were utilized. The experimental data obtained from these exposures indicates that radiation effects can be measured using a laser scanning confocal microscope (LSCM) for fluorescence imaging, where the observed effects correspond to dose and particle energy. Chemical analysis of irradiated films revealed that spirobenzopyran is converted to a different chemical species, which has not yet been identified. Future plans include continued chemical analyses to gain understanding of the chemical processes taking place, as well as the use of near field scanning optical microscopy (NSOM). The resolution of

NSOM exceeds that of LSCM and may be sufficient to resolve individual particle tracks, and thereby provide means to identify particle type.

The exposure of space crew to ionizing radiation poses a significant health hazard. Areas of particular interest include providing adequate dosimetry to crew members and understanding the complex radiation environment during mission in space. The exotic radiation environments that are present during space flight pose a unique dosimetry problem. These radiation fields may contain a variety of charged particle types, in particular HZE particles, having a broad energy spectrum. Currently, there is no radiation dosimetry method that has the combination of energy response and sensitivity to meet the needs of a complete crew dosimeter for space radiation environments. The lack of adequate dosimetry may result in unnecessary radiation exposure of humans in space. This project is directly related to NASA's space radiation program, and will enable us to establish the scientific basis for the radiation protection of humans engaged in the exploration of space. The development of effective dosimetry for space environments is essential for radiation protection and for advancing our understanding of the mechanism of radiobiological effects in humans.

FY97 Publications, Presentations, and Other Accomplishments:

Emfietzoglou, D. and Moscovitch, M. "Theoretical basis for solid state microdosimetry using photochromic alterations" in "Microdosimetry, An Interdisciplinary Approach." Edited by: Goodhead, D., O'Neill, P., and Menzel, H. The Royal Society of Chemistry, Cambridge, UK, pp 379-382 (1997).

Moscovitch, M. and Emfietzoglou, D. "Simulation of radiation effects on three-dimensional computer optical memories." J. Appl. Physiol., Vol.81, No.1, 58-69 (January 1, 1997).

Phillips, G.W., Mueller, S.C., and Moscovitch, M. "Investigation of 3-D ORAM material for space radiation dosimetry." Eighth Annual Space Radiation Health Investigators Workshop, Brookhaven National Laboratory (May 2, 1997).

Dosimetry for Populations Residing Near Techa River

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Funding:

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Initial Funding Date: 1997	Expiration: 2000
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$150,000	
Joint Agency Participation: Department of Energy	

Task Description:

Persons traveling in space can accumulate fairly large doses of radiation, up to several Sv, at low to moderate dose rates. In general these dose rates are low enough so that deterministic effects can be avoided, although shielding may be necessary. An important question, however, is the stochastic effects (induction of cancer and genetic defects) of these doses. Most radiation-risk estimates are based on the survivors of the atomic bombings on Japan, events which produced nearly instantaneous doses. It is hoped that stochastic effects would be less probable for lower dose rates, but few opportunities have been available to examine this question in humans.

The Mayak Production Association (MPA) was the first Russian site for the production and separation of plutonium. This plant began operation in 1948, and during its early days there were very high occupational doses as well as technological failures that resulted in the release of large amounts of waste (about 10^{17} Bq of liquid wastes) into the rather small Techa River. Residents along the Techa River were exposed to external radiation, and they ingested foods contaminated with ^{90}Sr and other radionuclides. The "Techa River Cohort" has been studied for several years by scientists from the Urals Research Centre for Radiation Medicine (URCRM).

The purpose of this project is to improve the dose-reconstruction system for the Techa River Cohort that has been under development for many years by Russian scientists at the URCRM. This, and the companion epidemiologic studies, are deemed to be unique and important, as members of the Techa River Cohort received red bone marrow doses of up to 3 Gy, but at low-to-moderate-dose rates. An increase in leukemia and cancer mortality has already been noted for this population, and further study should allow the evaluation of dose-rate-reduction factors for this situation.

The current dose-reconstruction system is grounded firmly on whole-body counts for half of the members of the cohort (for the evaluation of internal dose, which was mainly due to incorporated ^{90}Sr and ^{89}Sr) and on direct measurements of external gamma-exposure rates. Validation studies with thermoluminescence studies of natural dosimeters and of electron paramagnetic resonance of teeth are also being performed.

The specific aims of this project are to develop improvements in the existing dosimetry system for members of the Techa River Cohort by providing more in-depth analysis of existing data, further search of existing records for useful data, model development and testing, evaluation of uncertainties, verification of procedures, and validation of current and planned results. The purpose of the enhanced dose reconstruction is to support companion epidemiologic studies of radiogenic leukemia and solid cancers.

Studies of the possible effects of radiation on those exposed to the releases to the Techa River were started in Russia in the 1950s. Russian and United States scientists have been involved in collaborative research programs since 1995. The results of the feasibility study were published in 1996, and approval was received for the three-year project in February 1997. It is important to note that this project is a cooperative and collaborative activity of several US (DOE, EPA, and NASA) and Russian (Ministries of Health and Emergency Affairs) agencies; work is performed in close cooperation with additional work sponsored by the European Commission and other foreign groups. During the remaining part of FY1997 progress was made in several directions.

One major aspect of the project is to extend and validate the more than 15,000 measurements of ^{90}Sr in the whole body by use of a unique bremsstrahlung counter. This counter, however, is very old and was calibrated with an anthropomorphic phantom (which is no longer available) only at the beginning of its life. A major activity now underway is to upgrade this counter with new detectors and electronic systems. Before this is done, it is essential to recalibrate the old detector and to be able to calibrate the new system. The design of a new anthropomorphic phantom was completed during FY97, and the phantom is currently being manufactured. Funding was also provided for the upgrade of the counter system, and steps are underway for its acquisition. In the meantime, more persons continue to be counted or recounted in the system. Results derived from this unique body of data are also being used to refine models of ^{90}Sr metabolism in humans.

Another important aspect of the improvement process has been to check the consistency of available source term data and historical monitoring data; a major purpose is to confirm the limited information available on the source term and to develop an empirical model of the dependence on distance downstream of the radionuclide composition of river water and to link radionuclide concentrations in water and bottom sediments with gamma-exposure rates on banks and near the river. This work was completed in FY1997.

Many ways are being sought to verify independently the calculated doses. These involve a variety of recently developed instrumental techniques of dose reconstruction. Samples of bricks have been taken from now abandoned buildings, and the quartz inclusions have been used as thermoluminescent dosimeters. Samples of teeth have been taken, as the opportunity arises from extractions performed for health reasons, and used to measure accumulated dose by the technique of electron paramagnetic resonance. This technique, newly perfected for the purpose of dose reconstruction, has provided extremely valuable results. The pattern of external dose with distance downstream has been confirmed. Completed this year was a feasibility study on expanding greatly the scope of these investigations. This expanded study will focus on those living in the upper part of the Techa River who received the larger doses.

In the meantime work continues on a broad front of investigations. These include:

- Accumulation of family histories of lifestyle and source of drinking water (river vs. well).
- Study of the variations in gamma-exposure rate according to distance from river and the effects of location and time spent in streets, gardens, and homes.
- Study of organ-specific doses from external gamma exposure.
- Development of appropriate metabolic and dosimetric models for all radionuclides of interest.
- Development of a system to describe accurately the uncertainties (systematic bias and random errors) in all models and measurements and to propagate such uncertainties through to the final results with proper allowance for correlation structures within the data.

- Development of the improved Techa River Dosimetry System to provide doses for each individual member of the cohort and the uncertainty in that dose.

This project addresses the key question of the health risks of exposure to high doses of radiation at low-to-moderate dose rates. In this particular case, it is clear that a "space benefit" would be derived from studies on Earth if it could be demonstrated convincingly that there is a dose-rate amelioration effect in humans. Should this be true, the "Earth benefit" could be the relaxation of radiation-protection standards, including the concept of "as low as reasonably achievable." At the same time, from data received so far, this dose-rate amelioration effect may be much smaller than hoped, but it is important to define it well. Radiation exposure may well be one of the more critical limiting factors in space travel.

FY97 Publications, Presentations, and Other Accomplishments:

Degteva, M.O., Tolstykh, E.I., and Kozheurov, V.P. "Retrospective dosimetry related to chronic environmental exposure." Proc. of Int. Workshop "Intakes of Radionuclides: Occupational and Public Exposure," Avignon, France (September 15 - 18, 1997).

Tolstykh, E.I., Kozheurov, V.P., Vyushkova, O.V., and Degteva, M.O. "Analysis of strontium metabolism in humans on the basis of the Techa River data." *Radiat. & Envir. Biophysics*, Vol. 36, 25-29 (1997).

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Note: This entry represents nine individual tasks with nine principal investigators.

Funding:

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Initial Funding Date: 1997	Expiration: 2001
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$454,797	
Joint Agency Participation: NIH	

Task Description:

Overview Summaries for Nine Grant Applications Funded by the Joint NCI/NASA RFA (CA 96010) "Mechanisms of Genomic Instability from the Exposure of Mammalian Cells to High-LET Radiations." Initiated September 30, 1997.

**Robert Ullrich, Ph.D., University of Texas, Galveston, TX
 "Genomic Instability in High-LET Carcinogenesis"**

The possible susceptibility of mouse-mammary precursor epithelial cells *in vivo* to high-energy (HZE) or alpha irradiation that results in radiation-induced genomic instability and its consequences on malignant transformation are highly relevant to RFA objectives in order to explore the basis for tissue and cell-type sensitivities in the intact animal. Dr. Ullrich will determine the capacity of high-atomic-number, HZE iron particles (a major component of cosmic radiation), high-energy protons (a major component of solar flares), or alpha particles (a surrogate for radon). He will test the hypothesis that genomic instability is one of the first events expressed in irradiated cells *in vivo*, and is correlated with the subsequent occurrence of mutations in p53 and the loss of cell-cycle regulation in clones that display cytogenetic instability. These effects will be related to the acquisition of a mutator phenotype, and the capacity for DNA double-strand-break (DSB) repair *in vivo*.

William Morgan, Ph.D., University of California, San Francisco, CA
“Mechanisms of High-LET-Induced Genomic Instability”

This application will use human chromosomes in hamster/human hybrid cells as targets for the induction of genomic instability by HZE iron particles, high-energy protons or alpha particles. It will test several significant hypotheses, including the following: (1) these high-LET radiations induce chromosomal instability in both log-phase and confluent cells; (2) cells that show chromosomal instability subsequently demonstrate a mutator phenotype; and (3) both nuclear and cytoplasmic as well as nuclear ‘critical’ targets are involved in the induction of genomic instability. This application demonstrates a sophisticated use of molecular cytogenetics for the analysis of DNA sequences thought to be involved in the onset and transmission radiation-induced cytogenetic instability (e.g., progressive expression of DNA deletions, amplifications and rearrangements of telomere-like DNA sequences). The occurrence of mutations in unstable DNA sequences will be compared with the timing of expression of cellular and biochemical endpoints thought to be associated with radiation-induced instability (e.g., the acquisition of a heritable mutator phenotype, DNA double-strand-break capacity).

Helen Evans, Ph.D., Case Western University, Cleveland, OH
“Induction of Genomic Instability in Human Lymphoblasts”

The main hypothesis stated by Dr. Evans is that more than one molecular mechanism and thus more than one critical genetic target may be involved in the induction of genomic instability in human cells exposed to various high-LET radiations, including HZE iron particles and alpha particles. This may help to explain the unusually high rates of heritable genomic instability observed in mammalian cells exposed to high-LET radiations. This application will first identify and then analyze unstable clones for defects in DNA repair, e.g., DNA-polymerase fidelity, the fidelity of chromosomal segregation, and regulation of cell cycle progression. Mutant clones in these various categories will be characterized for the acquisition of heritable mutator phenotype and possible relationships to defects in DNA repair. Both this and the previous application (Morgan) address the possibility of multiple cellular targets for radiation genomic instability. However, Dr. Evans will focus on the possible role of DNA repair/replication systems rather than highly sensitive DNA sequences or membrane targets for induction of radiation instability.

Eric Hall, Ph.D., Columbia University, New York, NY
“High-Energy Ions and Genomic Instability”

The cumulative effect of multiple exposures of human cells to high-LET on genomic instability and its relationship to the eventual occurrence of malignant transformation of human cells is unknown. This application studies the possibility that heritable genomic instability damage from previous exposures of human cells to HZE irradiation increases the transforming efficiency from subsequent exposures to other forms of ionizing radiations (e.g., high-LET neutrons, high-energy protons, or low-LET xrays). Based on known responses of human cells to HZE, many progeny of surviving irradiated cells can be expected to transmit genomic instability at high frequencies to their decedents and thus are likely to be subsequently exposed to these other space-related ionizing radiations. This issue is highly relevant to the RFA since it has been estimated that 10% to 30% of *all* of the cells in an astronaut’s body will be traversed by at least one HZE particle with a charge greater than neon during a 2-year Mars space flight.

Joel Bedford, Ph.D., Colorado State University, Fort Collins, CO
“HZE Radiation-Induced Chromosome Instability”

The main focus of this application is to study the cytogenetic basis for the high frequency of delayed chromosomal instability observed after exposure of primary human fibroblasts cells to HZE iron or alpha particles. The alpha-particle studies will make use of a microbeam apparatus that can deliver a calibrated dose of radiation to a designated cellular location or cell compartment (e.g., cytoplasm, nucleus) and thereby gain insight on the cellular locations of critical targets for genomic instability induced by this type of high-LET radiation. Mendelian dominance/recessiveness segregation relationships for radiation-induced genomic instability will be

determined among clonal descendants from cells that survive irradiation with HZE iron or alpha particles. This application also will address the important issues of dose-response and the effect of oxygen tension on induction of HZE iron- or alpha-particle induced genomic instability in human cells.

Thea Tlsty, Ph.D., University of California, San Francisco, CA
“Mechanisms of Radiation-Induced Genomic Instability”

In this application, Dr. Tlsty will study mechanistic relationships between cell-cycle checkpoint expression (or lack of it), homologous DNA recombination, and the induction of genomic instability by HZE-iron particles. She will make use of novel human cell lines that she has developed that have an altered capacity for expression of the cell-cycle checkpoints (G1, S, and M) and capacities for homologous DNA recombination activity. Her primary hypothesis is that high-LET radiation induces unstable chromosome rearrangements in irradiated normal cells via the activation of a homologous recombination enzyme system. This enzyme complex, recently discovered by Dr. Tlsty *et al.*, includes hhRAD51 as a component and appears to be suppressed in the unirradiated normal human cells, although it is commonly expressed at high levels in tumor cells that have lost genomic control. Dr. Tlsty's application is complementary to the previous application (Dr. Chen) with emphasis on radiation-induced homologous DNA recombination repair rather than DNA end joining and DSB repair.

David Chen, Ph.D., University of California, Los Alamos National Laboratory, Los Alamos, NM
“Roles of Human Recombination in Genome Instability”

This application addresses the possible biochemical mechanisms responsible for the elevated rates of DNA recombination observed in mammalian cells exposed to high-LET radiations and associated with the persistence of radiation genomic instability among their progeny. In this application, Dr. Chen postulates that the human equivalents of the yeast RAD51 and RAD52 genes (hhRAD51, hhRAD52) have critical roles in maintaining genome stability and that the loss of one or both of these genes exacerbates the onset and genetic consequences of radiation-induced genomic instability. He implies that these two genes are likely to play a significant role in radiation-induced DSB repair, and that the loss of normal DSB-repair capacity occurs during the onset of HZE and alpha particle-induced genomic instability. Although the success of this application will largely depend on the ability to maintain the cell's viability while manipulating the expression of these enzymes (e.g., through use of single or double mutants or mRNA antisense suppression of gene expression), this grant was identified by the study section as high scientific risk, high benefit because of its potential to advance critical knowledge in the mechanisms and consequences of high-LET-induced DNA recombination in human cells.

Antone Brooks, Ph.D., Washington State University, Pullman, WA
“*In Vivo* Induction and Repair of Genomic Instability”

Dr. Brook's application will use the rat model to study the susceptibility of deep-lung and tracheal epithelial and hematopoietic precursor cells to genomic instability induced by high- and low-LET radiations. The main hypotheses in this application are the following: (1) genomic instability is related to the amount of primary DNA damage from irradiation; (2) genomic instability can be induced *in vivo* and is directly related to sensitivity for cancer induction; and (3) the DNA lesions responsible for high-LET-induced genomic instability are lost as a function of time. The dose-response relationships based on the induction of micronuclei by HZE iron particles will be generated and compared with those generated by low-LET xrays; this will be indicative of the relative biologic effectiveness of these radiations and of their capacity, as a function of LET, to induce primary DNA damage. The long-term transmissible cytogenetic manifestations will be monitored by fluorescence *in situ* hybridization.

Amy Kronenberg, Ph.D., University of California, Lawrence Berkeley Laboratory, Berkeley, CA

“High-LET Radiation and Genomic Instability in Human Cells”

Dr. Kronenberg's application focuses on the relationship between radiation-induced apoptosis and the suppression of HZE- and proton-induced genomic instability. Recently, subsequent to the review of this RFA, she recently has provided the first experimental evidence that *low* fluences of HZE iron particles induce delayed apoptosis in cultured human cells, and that radiation-induced apoptosis can inhibit the onset of radiation-induced genomic instability. This important observation, related to the major objectives of this application, suggests that a critical role of radiation-induced apoptosis is to prevent replication of somatic cells expressing heritable genomic instability (e.g., suppression of apoptosis by overexpression of the BCL protooncogene leads to a significant increase in expression of a mutator phenotype and the rapid accumulation of genomically unstable clones). Dr. Kronenberg's proposal to the RFA is the only application to propose studies on molecular relationships between apoptosis and HZE-induced genomic instability.

These applications were jointly funded by NCI and NASA on September 30, 1997, thus progress has been minimal for 1997. Funding is by the NIH R01 mechanism for four years for each application except for one (Dr. Brooks) which will be funded three years.

These applications address basic mechanisms of genomic instability induced by HZE, high energy protons, and other forms of ionizing radiation likely to be encountered during prolonged space flight. This information will be important in estimation of risk due to cancer or heritable genetic damage by astronauts exposed to such radiations and may feed into engineering calculations for levels of shielding that will be required by astronauts during space flight operations.

Effects of Exposure to Heavy Particles

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Funding:

UPN/Project Identification: 199-45-17-16

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Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$26,511

Task Description:

Future missions in space (such as a mission to Mars) may involve long-term travel beyond the magnetic field of the Earth, subjecting astronauts to radiation hazards posed by solar flares and galactic cosmic rays. The objectives of the present proposal are to describe and characterize heavy particle-induced behavioral and neurochemical deficits, determine their underlying causes, and develop approaches to minimize such deficits. To achieve these objectives, we have been using behavioral and neurochemical models that have been previously shown to be sensitive to exposure to radiation, and which may provide a basis for defining the effects of exposure to heavy particles on brain functioning and related behavior.

The initial experiments involved studying the effects of exposure to one GeV/n iron (^{56}Fe) particles on: 1) dopamine-mediated motor behavior, by studying upper body strength measured by a wire suspension task; 2) oxotremorine enhanced dopamine release from striatal tissue using HPLC; and 3) the behavioral toxicity of one GeV/n iron particles, measured using the conditioned taste aversion paradigm. The results of these experiments showed that exposure to 1 GeV/n ^{56}Fe particles produce deficits in these endpoints similar to those produced by exposure to 600 MeV/n ^{56}Fe particles. However, exposure to 600 MeV/n particles elicited neurochemical deficits and behavioral changes at significantly lower doses than were required to produce those changes following exposure to 1 GeV/n particles.

Continuing experiments are designed to determine the cellular mechanisms that mediate the neurochemical and behavioral changes produced by exposure to low doses of one GeV/n iron-56 (^{56}Fe) particles and to examine the range of dopamine mediated behaviors that may be affected by exposure to these iron particles.

The research associated with this project requires access to the Alternating Gradient Synchrotron (AGS) at Brookhaven National Laboratory (BNL). The AGS has not been available for Life Sciences research since October/November 1996. The results of the experiments run at that time were described in the report submitted in January 1997.

We are currently preparing for the 1997 run of the AGS which is scheduled to begin on October 17, 1997. The experiments which we expect to complete include:

- 1) Long-term effects of exposure to ^{56}Fe particles on amphetamine-induced taste aversion learning. This experiment is designed to determine whether the behavioral changes observed immediately following exposure are observed 2-3 months later.
- 2) Effects of exposure to ^{56}Fe particles on membrane fluidity and viscosity. This experiment will utilize s-adenosyl methionine to determine whether or not changes in membrane structure mediate the changes in dopaminergic function observed following exposure.
- 3) Effect of exposure to ^{56}Fe particles on free radical activity in the brain. This experiment will involve the analysis of 2,3- and 2,5-dihydroxybenzoic acid to determine the role of oxidative stress in heavy particle-induced changes in neurochemical functioning.

We also anticipate running pilot experiments which will provide a basis for continuing research on the behavioral and neurochemical effects of exposure to heavy particles. These include experiments designed to explore:

- 1) The possible effects of exposure to ^{56}Fe particles on dopaminergic reinforcement mechanisms by studying amphetamine-induced conditioned place preference learning.
- 2) The effects of exposure on hippocampal-mediated learning by studying the performance of irradiated rats in a water-maze.
- 3) A possible role for diets rich in free-radical scavengers to ameliorate the neurochemical effects of exposure to heavy particles.
- 4) Possible morphological changes in the brain produced by exposure to heavy particles using immunocytochemical techniques.

The research which we have conducted previously has shown that exposing young rats (less than 3 mo. old) to low doses of heavy particles (^{56}Fe , 600 MeV/n) has shown that the neurochemical deficits produced by this exposure are similar to those that are observed in aged rats (24 mo. old). The research which we have just completed at BNL indicates that similar deficits are observed following exposure to one GeV/n ^{56}Fe particles as well. Thus, exposing rats to low doses of ^{56}Fe particles provides a way to accelerate aging in experimental animals with respect to certain brain and behavioral parameters so that experimenters do not have to wait for 24 months to obtain old animals.

The research program is designed to understand the mechanisms by which exposure to heavy particles (primarily ^{56}Fe) produce their effects on brain and behavior. Although the impetus for the research is to understand and minimize the effects of exposure to heavy particles on astronauts, the research program necessarily has implications for the understanding of the natural aging process. Because exposure to ^{56}Fe particles may produce accelerated aging, this research is also indirectly concerned with the basic processes underlying the biology of aging. Similarly, because the ultimate goal of the research program is to develop interventions to minimize the effects of exposure to heavy particles on astronauts, the interventions may also prove useful with the natural aging process.

FY97 Publications, Presentations, and Other Accomplishments:

Joseph, J.A., Erat, S., and Rabin, B.M. "Selective efficacy of space-like radiation effects (^{56}Fe particles) on muscarinic neurotransmitter sensitivity and motor behavior." *Adv. Space Res.* (In Press).

Joseph, J.A., Shukitt-Hale, B., Rabin, B.M., and Erat, S. "Oxidative stress in the CNS and cosmic radiation (^{56}Fe particles): Implications for immediate or delayed behavioral performance deficits." Presented at the 8th Annual Space Radiation Health Investigators Workshop, Upton, NY (April 29 - May 3, 1997).

Rabin, B.M., Joseph, J.A., and Erat, S. "Effects of exposure to different types of radiation on behaviors mediated by peripheral or central systems." *Adv. Space Res.* (In Press).

Rabin, B.M., Joseph, J.A., Shukitt-Hale, B., and Erat, S. "Effects of exposure to 1 GeV/amu ^{56}Fe particles on amphetamine-induced taste aversion learning." Presented at the 8th Annual Space Radiation Health Investigators Workshop, Upton, NY (April 29 - May 3, 1997).

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Funding:

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Initial Funding Date: 1996	Expiration: 1997
Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$3,000,000	
Joint Agency Participation: NIH	

Task Description:

The primary objective of the Cooperative Research in Proton Space Radiation activity is to establish and maintain facilities and a supporting academic program to promote investigations into the health effects of charged particle radiation with emphasis on protons which represent the principal radiation hazard to astronauts. Loma Linda University operates a facility for therapy of cancer and other diseases using accelerated protons from a synchrotron which is located within the medical center. Associated with the synchrotron are treatment rooms and all clinical services relevant to radiation therapy. Also associated with the synchrotron are an experimental area ("research room") which can receive a proton beam and an adjacent staging laboratory from which the accelerator can be operated and experiments may be configured prior to irradiation. Close to the accelerator is the new Chan Shun Pavilion, a wing of a research building whose first floor has been designated for a radiobiology research program with capabilities for modern cellular, molecular, and *in vivo* biology studies. Included in this structure is a laboratory dedicated for the use of visiting scientists whose research requires access to proton beams. The new facility was approved for occupancy in late April, 1997.

In year one of the cooperative agreement, a basic beam line was designed to bring protons of from 40 to 250 MeV to the research room for experimental work while not interfering with patient treatments. The design was based on a science requirements document approved by scientists performing charged particle experiments and subjected to a design review by accelerator physicists from several national laboratories and universities. The new beam line will provide for flexible delivery of proton beams at doses, dose rates, energies, field sizes, and field uniformities that are adequate for many biology, physics, and materials science experiments. Components of the beam line have been procured and installation and commissioning is scheduled for completion by March 1, 1998. Enhancements to the beam line are planned for future contract years. Protons are currently available for experiments only after hours under a limited set of conditions in treatment areas. A Cobalt-60 irradiator has

been installed to provide gamma rays for control experiments.

The radiobiology laboratories have been commissioned following procurement and installation of equipment and supporting facility modifications. A dedicated laboratory for visiting investigators has also been commissioned and configured to operate in parallel to the main laboratory suite to enable visitors to work independently. The staging laboratory adjacent to the research room is under development with most equipment items received. A series of customized carts have been fabricated to transport cells and animals under controlled environmental conditions between locations. Training on analytical instruments is ongoing and provisions for training visitors are being made.

Cell, molecular and animal-based research activities began in early September. These investigations focus on the radiobiology of cultured epithelial cells, human glioma cells, nematodes, nervous system microvasculature, and the application of cytokines to tumor control. Initial efforts have been to validate the operational status of equipment and facilities.

Contractual Matters

Cooperative Research Agreement NCCW-98 was entered into between NASA and Loma Linda University for the purpose of establishing research programs and facilities that support biological and physical studies into the effects of charged particle radiation with emphasis on protons because these radiation species represent a significant health risk to space travel. The grant was awarded for the period June 1, 1996 through May 31, 1997 and later extended at no cost through September 30, 1997 due to delays in building construction at Loma Linda.

Facility Overview

The Loma Linda University Proton Treatment Facility (PTF) and Cancer Research Institute (CRI) Radiobiology Laboratories are the principal sites of radiation research activities supported by this agreement. The PTF is a synchrotron which delivers beams of protons to five therapy target areas and a research room all located in the Loma Linda University Medical Center (LLUMC). Adjacent to the research room is a staging room and in the nearby animal care facility is a room configured for a radioisotope or X-ray irradiator required as a control radiation source for protons. Four hundred feet away and connected to the Medical Center PTF is the CRI building. The first floor of the CRI building houses six (750 ft² each) investigator laboratory modules and a series of core laboratories for multi-user instruments. An additional two-module (1500 ft²) laboratory is maintained exclusively for visiting scientists. Offices and a conference room are situated next to the laboratory suite.

The multiyear construction process for the CRI building concluded in April, 1997 when the building received occupancy permission from the city of Loma Linda. This was some six months later than anticipated at the time of initial funding. Offices were equipped during April (LLU funding) and the process of modifying and equipping the laboratory suites with NCCW-98 funding began approximately May 1, 1997. The first investigator, Prof. Daila Gridley, relocated her laboratories to the CRI in August and research activities commenced in September.

The heavily shielded animal facility room needed to house a gamma or X-ray irradiator was readied by removal of an obsolete gamma ray therapy machine and a new Eldorado Model 8 Cobalt-60 irradiator was installed in September 1997.

At the beginning of the funding period, the research room was intermittently able to receive a raw beam of protons to a vacuum line located inside the room and controlled remotely from the main control room. All experimental work under these conditions was limited to beam configurations designed for patient treatment and restricted to late night and occasional weekend opportunities. Environmental control and local biological laboratory handling conditions were highly limited. In order to deliver a beam at any available energy with uniform intensity over target areas matching experimental equipment dimensions a beam line needed to be

designed and installed. A beam line consists of an integrated system of precision mechanical mounting fixtures, vacuum systems, magnetic lenses, electronic detectors, scatterers, computers, electronic control console and interfaces to the existing therapy system, alignment lasers, environmental controls, and customized software to control the system. The research beam line must operate safely without interfering with PTF patient therapy operations.

To define the scientific requirements for the beam line, a working group consisting of physicists, engineers, and biologists was convened to draft a science requirements document during a series of half-day meetings in the Spring of 1997. This was circulated for review by the NASA space radiation health program investigators at their 8th annual meeting. The revised science requirements document served as the basis for a preliminary engineering design by the systems engineering firm, Optivus Technology, Inc., which participated in the construction of the PTF and currently maintains the synchrotron. In parallel to the preliminary design effort, a series of beam line components matching those of the therapy system was procured to reduce the impact of long lead time fabrication. The preliminary design was formally reviewed in July by physicists, biologists, and engineers from LLU, Lawrence Berkeley Laboratory, and Brookhaven National Laboratory. The review and preliminary design have been combined to generate a detailed design specification as well as hardware and software test plans. The final detailed engineering, procurement, installation and testing phase will be funded from year two of the agreement with a target date of March 1, 1998 for commissioning. The staging laboratory adjacent to the research room is currently being upgraded to support appropriate biological support equipment.

Capital Equipment and Core Facilities

A major effort commencing at the beginning of the funding period was the identification, procurement and installation of major and minor laboratory equipment to support modern molecular, cellular, and animal biology. The major equipment items secured are organized into the following conceptual core laboratory groupings that are generally co-located. These items are available to visiting investigators working in the dedicated NASA Visitors Laboratory.

- Dishwashing and Autoclave Laboratory
- Steam Autoclave
- Automatic Glassware Washer
- Automatic Glassware Drier
- Associated Carts and Racks

- Biochemical Preparation Laboratory
- Walk-in Cold Room
- Lyophilizer
- Vacuum Centrifuge
- Chest Ultralow Freezer
- Three Preparative and Analytical Ultracentrifuges & Rotors
- Ice Machine
- Liquid Nitrogen Storage System
- Dry Ice Chest & Dry Ice Block Maker

- Animal Procedures Laboratory
- Surgical Table
- Anaesthesia System
- Stereotactic Frames
- Surgery Tools, Tagging & Implant System
- Laminar Hood
- Two Cage Carts

- Radioisotope Laboratory

- Liquid Scintillation Counter
- Multiformat Scintillation Counter / Luminometer
- Dedicated Chemical Hood
- Shielded Storage Areas

- Darkroom
- Gel & Plate Imaging System & Illuminators
- Developing Sink & Slot Hood
- Film Drawers
- Automatic X-ray Film Developer

- Analytical Instrument Laboratory
- Flow Cytometer
- Phosphor Imager
- Spectrophotometer
- Multiwell Spectrophotometer / ELISA Reader
- Multiwell Spectrofluorometer / ELISA Reader
- Capillary Electrophoresis System

- Cell Preparation Laboratory
- Laminar Hood
- Elutriation Centrifuge
- Coulter Counter & Analyzer
- Automated Media Sterilizer / Plate Pourer
- Inverted Microscopes
- CO₂ Incubators

- Active Storage Room

- Microscopy Laboratory
- Laser Scanning Cytometer
- Research Microscope (DIC, Fluorescence, Phase)
- Laser Microbeam Cell Ablation System
- Videotape Recorder, Camcorder
- Cooled CCD camera
- Image Processing Computer Workstation
- Dye Sublimation Printer

General Laboratories & Visitor Laboratories

- Miscellaneous Water Baths
- Ultrapure Water System
- Biogard Laminar Flow and Chemical Hoods
- Electrophoresis Equipment and Power Supplies
- PCR Thermocycler
- Ultralow and -20C freezers
- Refrigerators
- Miscellaneous Incubators for Cells and Microorganisms
- PH Meters
- Balances
- Conductivity Meter
- Osmometer
- Stereo, inverted and upright compound microscopes
- Mixing Devices: vortexers, stir plates, rotators
- Small autoclaves and centrifuges

- Miscellaneous shop & hand tools
- Workbench
- Pipetting Devices
- Heating plates and dry blocks
- Cell disruption equipment, rotary and ultrasonic
- Miscellaneous glassware & hardware
- Laboratory chemicals
- Laboratory Carts
- Two customized carts for transport of cell incubators and cell biology workstation to gamma irradiator and proton treatment areas
- Cryogenic storage and handling vessels

Research Activities

Research activities commenced in September, 1997. The initial staffing consists of two permanent investigators (G. Nelson and D. Gridley), one sabbatical investigator from the J. Pettis Veterans Administration Medical Center in Loma Linda (L. Green), and one emeritus investigator from LLUMC Department of Radiation Medicine (J. Archambeau). Three research assistants, one Research Fellow, and three graduate students are supporting this staff. The research focus is on the effects of radiation on epithelial cell functions, microvasculature organization in the rat nervous system, cytokine expression, and nematode genetic alterations. In addition to assessment of scientific issues, the initial studies provide verification of facility and equipment function.

The proton synchrotron which is being upgraded for research into the biology and physics of protons is used as a therapy device for treatment of a variety of cancers (*e.g.*, prostate, head and neck, brain) as well as vascular abnormalities in the brain (arteriovenous malformations). Its delivery of radiation doses is spatially more precise than conventional X-ray or gamma ray systems allowing more effective treatment to body sites nearby critical organs. This same positional accuracy also enables research protocols in radiobiology which cannot be achieved with traditional ionizing radiation sources. An example is the localization of radiation to portions of the rat brain without damaging the oral cavity in order to study the influence of the vasculature and supporting glia on the radiation response of the central nervous system (CNS). Such investigations are critical to understanding the response of the CNS in pediatric brain tumor treatment.

The structured pattern of energy deposition by protons and other charged particles produces qualitatively and quantitatively different damage to cell membranes and DNA than X-ray and gamma ray photons. Clusters of damage on a molecular scale are the result and test the adaptive and repair responses of cells thereby revealing new details of cell regulatory pathways. Management of these responses presents a possible avenue to improved radiation treatment. The kinetics and fidelity of these repair processes affects the radiation exposure risk assessments for astronauts travelling outside the Earth's atmosphere, occupational radiation exposures on Earth, and environmental exposures to radon.

The proton synchrotron is also used in assessing the performance of electronic devices which are used in space craft and in validating the transport of radiation through shielding materials. Electronic parts must be "hardened" to resist reversible or permanent defects which are caused by the traversal of charged particles found in space. These single event "upsets" and "latch-ups" can have devastating effects on computer-controlled guidance and control systems. Defects in manned spacecraft pose substantial safety hazards to crews while defects in weather and communication satellites pose potential commercial losses. Parts testing and shielding testing enable improvements in manufacturing processes.

In summary, the ability to simulate a substantial component of the space radiation environment (protons) enables cost effective assessment of biological and physical system responses to radiation. Biological information gathered in pursuit of space radiation health issues is directly applicable to radiotherapy and *vice versa*.

Risk Management Strategies During Solar Events

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No Co-Is Assigned to this Task

Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$60,641

Task Description:

The natural radiation from galactic cosmic rays (GCR) and solar particle events (SPEs) will pose a serious health risk to humans during missions to Mars or the Moon. The opportunities and limitations of astronauts on these missions will be constrained by the radiation environment and NASA's response to it.

NASA recognizes both an ethical and legal responsibility to minimize the acute and long-term risks to astronauts during space flight. There is an extensive program underway to collect statistically meaningful data to understand the fundamental biological impacts of the radiation environment. These data can be directly related to the well-characterized incident, slowly varying, galactic cosmic ray flux. There is greater uncertainty in predicting and characterizing the rapidly changing solar particle radiation which, over time periods of hours to days, can produce particle fluences comparable to mission-long GCR fluence.

Work by this investigator in an earlier grant (NAGW-4166) systematically identified the types of solar particle events (SPEs), the stages of a human Lunar or Mars mission when the events may occur, and the methods used to detect and forecast these events. Alternative scenarios were identified that represented the range of architectures that could be employed to mitigate SPE risk. The advantages and disadvantages of each scenario were discussed.

Based on the earlier results, we initiated a program to continue this research through a sequence of additional phases that will progressively add to NASA's ability to develop a comprehensive SPE risk management strategy. These subsequent phases examine in more detail the science, engineering, and operational requirements. The focus in each phase will be to identify the key issues and general approaches to address these issues, rather than to attempt to specify a particular solution.

During this time, the Principal Investigator, Dr. Ronald Turner, continued work on the general problem of the impact of solar particle events on human space flight.

Dr. Turner completed the ANSER technical report "Physics of Solar Particle Events," in November, 1996. This report is the first of a series of reports intended to prepare a comprehensive review of the basic physics, applied physics, forecasting, and operational options for a risk mitigation strategy for solar particle events during human missions to the Moon or Mars. The PI also prepared a draft of the ANSER technical report "Forecasting Solar Particle Events," (due for completion in November 1997.) This report is the second of the series.

The PI attended the NSF/NOAA workshop on Space Weather in Boulder, Colorado, on January 16-17, 1997, and a working session of the Solar, Heliospheric, and Interplanetary Environment Workgroup on January 18, 1997. Dr. Turner also attended the NASA JSC Workshop on Radiation Monitoring on Mars, February 13-14, 1997, which prepared NASA Life Science recommendations for the robotic precursor mission to Mars in 2001 and the NASA JSC Workshop on the Impact of SPEs on Design of Human Missions.

Dr. Turner researched and of reports intended to prepare a comprehensive review of the basic physics, applied physics, forecasting, and operational options for a risk mitigation strategy for solar particle events during human missions to the Moon or Mars.

Finally, Dr. Turner submitted a proposal for follow-on activities to continue to investigate the risk of Solar Particle Events to astronauts, to include detailed reports on the applied physics of the events as well as the operational and engineering implications to human missions beyond the Earth's magnetosphere. This grant, NAG5-3888, was awarded and is continuing the SPE analysis started under NAGW-4166.

Solar Particle Events (SPEs) pose a health risk to astronauts in space. A better understanding of SPEs serves to better define the nature of radiation the astronauts are exposed to. This, in turn, helps to interpret radiation exposure and related effects as measured in space. SPEs also affect the electronics of space-based systems, including systems that are routinely used to support terrestrial communication, weather forecasting, and navigation. Further research into predicting the timing and intensity of SPEs may help mitigate the risks to these critical systems.

FY97 Publications, Presentations, and Other Accomplishments:

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Turner, R.E. and Baker, J.C. "Solar particle events and the International Space Station." Presented at the IAA 12th Man in Space Conference (June, 1997).

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HZE Radiation Genotoxicity in Cultured Mammalian Cells

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Funding:

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Initial Funding Date: 1995 Expiration: 1998
Students Funded Under Research: 7 Post-Doctoral Associates: 3
FY 1997 Funding: \$ 129,794

Task Description:

Sojourners in deep space will be exposed to high energy (HZE) ionizing radiations. The evidence now available is that these radiations potentially induce genotoxic effects like cell death, mutation, and chromosomal aberrations. The last two effects are particularly worrisome since mutated cells are known to cause cancer. However, a much better understanding of the mutagenic potential of HZE irradiations is essential to making rational decisions about risks to space travelers. Our participation in a NASA NSCORT gave us access to HZE ⁵⁶Fe at LBL where some data were generated for the genotoxicity in the A_L human x hamster cell hybrid cell line which is, per unit dose, more sensitive to HZE-mutagenesis than any other *in vitro* cell line. Genotoxicity can, therefore, be studied at relatively low doses. But our resources via the NSCORT, while appreciated greatly, are quite limited. Additional funds have been requested to speed up and expand the work with HZE irradiations, using principally ⁵⁶Fe, 150 GeV/m at BNL. The A_L human x hybrid *in vitro* mutation assay will be employed to quantify HZE mutagenic activity and to identify enough mutants to be subjected to Southern and PCR analysis to define mutational spectra for low doses of HZE irradiations. The program would be expanded to include extensive molecular cytogenetic analysis of chromosomes of the A_L hybrid and of normal human fibroblasts. These data on chromosomal mutation and chromosomal aberrations are not now available and would help in understanding and predicting radiation risks of space travel.

Excellent progress was made in our studies of genotoxic effects inflicted on cultured mammalian cells by radiations of kinds likely to be encountered on Earth and by travelers in space. These data can be used to help predict health risks, especially from cancer, in exposed populations or individuals. Toward this end, we compared the lethal and mutagenic effectiveness (both the number of mutants and types of mutations) of radiations of various LET (linear energy transfer) including X- and ¹³⁷Cs-rays, 55 MeV protons, 32 MeV/amu

nitrogen ions, 14 KeV/ μm carbon (290 MeV/ μ), 90 KeV/ μm particles, and HZE-Fe, (600 MeV/amu, LET = 190 keV/ μm). These irradiations were carried out at the Department of Radiological Health Sciences, Colorado State University, Lawrence Berkeley Labs, Brookhaven National Labs, and at the National Institute of Radiological Sciences, Chiba, Japan. Mutation was measured at the S1 loci of A_L and the A_LC cells. These hybrid cell lines contain a single human chromosome, number 11. A gene at 11p13.5 encodes a human cell surface antigen, S1. This gene and antigen provide well-defined markers for mutation. Mutations involving millions of base pairs of DNA can be measured in A_L cells, but mutations causing the loss of the entire short arm of chromosome 11 or of the entire chromosome 11 are lethal to this cell line and escape detection. The A_LC hybrid was engineered so as to overcome this restriction on the size of detectable mutations which increases its sensitivity to mutation.

Expressed in terms of mutants per unit dose, the Relative Mutagenic Effectiveness (RME) for S1⁺ mutants in A_L was 1, 0.75, 0.75, 2.5, 2.0, 2.0, 10 for X-, ^{137}Cs -, protons, nitrogen, HZE-FE, and γ -radiations, respectively. But per equitoxic dose (e.g., LD₅₀) rather than per unit dose (e.g., Gy), these RME were 1, 0.9, 0.5, 0.6, 0.7, 0.6, 1.5, respectively. This latter way of expressing mutant yields more accurately reflects carcinogenic risk since it compares the fraction of surviving cells containing mutation, and which have, therefore, an increased potential to initiate tumors. The relative values of mutants per LD₅₀, yields in A_LC exposed to ^{137}Cs -, protons, and neutrons were the same as in A_L , although the for 150 of ^{137}Cs - in the former versus 1400 in the latter. We have also shown (5) that a low dose of ^{137}Cs - (3 or 4 cGy) reduces the mutant yield induced by a later dose, and alters the kinds of mutants induced as well.

Although the RME per equitoxic dose of these higher LET radiation was about like that of lower LET radiation, the mutant spectra for low and high LET were different; in general, the higher LET radiations induced more large, unstable mutations than lower LET radiations. Estimating risks will, then, require continued efforts to quantify mutation and to understand mechanisms underlying mutagenesis resulting from radiations of different qualities, delivered under different exposure conditions to cells with different metabolic states, particularly regarding radical- intercepting and DNA repair mechanisms.

The goal of our NASA-sponsored program is to provide data that will improve predictions of risk and reduce the incidence of radiation-induced genetic disease such as cancer in space travelers or exposed populations of individuals on Earth. We are using our sensitive, relevant, but relatively cheap mutation assays to provide data on risks of genetic diseases associated with exposures to radiation, or to any other DNA damaging agent that exists in the environment in space or on Earth. These studies are shedding light on mechanisms of mutagenesis and relationships of mutation to carcinogenesis. The sensitivity to mutation of A_L -based assay is allowing us to investigate effects of potential antimutagens/anticarcinogens at the low doses of mutagen where most human exposures occur using low, non toxic doses of antimutagen, and investigate genetic susceptibility, especially the role of reactive oxygen intercepting chemicals and DNA repair mechanisms.

FY97 Publications, Presentations, and Other Accomplishments:

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Hei, T.K., Wu, L., Liu, S., Vannais, D., Waldren, C., and Randers-Pehrson, G. "Mutagenic effects of a single and an exact number of alpha particles in mammalian cells." Proc. Natl. Acad. Sci. USA, 94:3765-3769 (1997).

Kraemer, S.M. Mutation studies in two newly constructed human-hamster hybrid cell lines. (Dissertation) Colorado State University (1996).

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Waldren, C.A., Vannais, D.B., Drabek, R., Gustafson, D.L., Kraemer, S.M., Lenarczyk, M., Kronenberg, A., Hei, T.K., and Ueno, A. "Analysis of mutant quantity and quality in human-hamster hybrid human-hamster hybrid A_L and A_L-179 cells exposed to ¹³⁷Cs gamma rays or HZE-Fe ions." *Adv. Space Res.* (In Press).

Radiation Anticarcinogenesis by Thiazolidine Prodrugs

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Funding:

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Students Funded Under Research: 1
FY 1997 Funding: \$177,867

Solicitation: 95-OLMSA-01
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Post-Doctoral Associates: 0

Task Description:

The long-term objective of the research is to develop nontoxic, radiation protectors which, when taken orally, will diminish the long-term genetic consequences of space radiation exposure. Available compounds which serve as effective radioprotectors cause nausea when taken orally and are cytotoxic at radioprotective concentrations. A class of thiazolidine radioprotectors has been developed at the University of Utah. These compounds are nontoxic at radioprotective concentrations. The specific aims of this proposal are designed to determine the capacity of these thiazolidine prodrugs to protect mammalian cells from the long-term genetic effects (e.g., mutagenicity and carcinogenicity) which result from exposure to space radiations. Mammalian cells (human mammary epithelial cells) will be exposed to gamma-ray (^{137}Cs) or proton irradiation in the presence and absence of thiazolidine prodrugs. Alternatively, previously irradiated cells will be exposed to thiazolidine prodrugs up to 24 hours after irradiation. The capacity of these compounds (present during or after irradiation) to protect mammalian cells from the induction of cytotoxicity (measured as single cell survival), chromosome damage (translocations measured by fluorescence *in situ* hybridizations of mitotic chromosomes with chromosome specific DNA probes), mutations (*hprt* locus mutagenesis measured as the production of 6-thioguanine resistance), and carcinogenesis (measured as induction of cell transformation) will be quantified.

We expect to identify thiazolidine prodrug concentrations which, when administered during or after irradiation, protect mammalian cells from the induction of deleterious genetic effects such as carcinogenesis. The future goals of this research will be to determine the utility of these compounds for animal radioprotection, and the potential for oral administration of these compounds to humans. If these compounds are found to be nontoxic at effective radioprotective concentrations, they will be indicated as useful radioprotectors for astronauts.

^{137}Cs -irradiated 184A1 cells exhibit a toxicity curve with a D_0 of 0.4 Gy and a D_{50} of 1.8 Gy. Radiation sensitivity is equivalent for both proliferating and confluent (non-growing) cells assayed either immediately or 24 hours after irradiation. Thus 184A1 cells exhibit little or no potentially lethal cell recovery during post-irradiation incubation. Exposure to 4 mM WR1065 or RibCys (a thiazolidine prodrug) for 90 minutes results in approximately 45% and 15% cytotoxicity, respectively, and a radiation protection factor (the ratio of the fractional survival of cells irradiated in the presence relative to the absence of the drug) of 4.5 and 1.4, respectively. Longer drug exposures prior to irradiation result in increased drug toxicity and reduced levels of radiation protection. 184A1 cells exhibit a background mutation frequency of approximately 3×10^{-6} (about 3

mutations per 10^6 viable cells) at the X-linked *hprt* locus. We are presently involved in determining the impact of thiolamine exposure on the survival of confluent 184A1 cells (a growth condition presumed to be a more realistic approximation of normal tissue cells) and on the radiation induction of *hprt* mutations.

We have initiated experiments to investigate potential mechanisms for thiolamine antimutagenesis. Two mechanistic routes by which thiolamine exposure might modulate radiation-induced mutagenesis and carcinogenesis might be 1) modulation of radiation-induced apoptosis, and/or 2) modulation of cellular repair processes. In the TB8.3 murine p53^{+/+} hybridoma, exposure to mM concentrations of WR1065, but not RibCys or Cysteine, induce apoptosis within 12 hours. In the human SK-N-SH neuroblastoma, which contains a cytoplasmically sequestered, wild-type p53 protein, a 90 min exposure to mM concentrations of WR1065 induces apoptosis within 24 hours. Paradoxically, a brief exposure to mM concentrations of WR1065, RibCys or cysteine inhibit radiation-induced apoptosis, while continuous post-irradiation exposure to mM concentrations of RibCys or cysteine enhance radiation-induced apoptosis. The results are consistent with brief exposures to these thiolamines reducing radiation effects because of their chemical capacity to scavenge radiation-induced radicals. Longer exposures to higher concentrations appear to activate either wild-type nuclear or cytoplasmically-sequestered p53, resulting in enhanced radiation-induced apoptosis. Thiolamine activation of the p53 stress response may simultaneously modulate downstream DNA repair processes, including recombinational repair, enhance the fidelity of this repair pathway, and thereby reduce mutagenesis. We are currently undertaking studies to examine the mechanism(s) by which thiolamines activate the p53 stress response, including analysis of changes in intracellular redox state and changes in nuclear transcriptional activity.

The primary goal of this research project is to develop nontoxic protectors which, when taken orally, will diminish the long-term genetic consequences of space radiation exposure. A natural extension of this research will be the utilization of compounds found to be effective protectors for reducing the genetic risk in humans exposed routinely or accidentally to ionizing radiations in the Earth environment. Nontoxic radioprotective compounds will be useful for reducing tissue toxicity and carcinogenesis in individuals exposed to ionizing radiation in their work environment. Additionally, nontoxic, radioprotective compounds will be useful for normal tissue protection during cancer radiation therapy, decreasing the risk and increasing the effectiveness of cancer radiotherapy.

The paradoxical nature of the interaction of thiolamines with radiation damage (e.g., radiation protectors under some exposure conditions and radiation sensitizers under others) indicates that the informed use of these compounds may also be used to radiosensitize some cancers while protecting normal cells during clinical radiation therapy. Our expectation is that a thorough investigation of the mechanism(s) by which thiolamines enhance radiation killing of tumor cells (e.g., the murine TB8.3 hybridoma and the human SK-N-SH neuroblastoma) will indicate appropriate thiolamines, appropriate concentrations of thiolamines, and appropriate exposure timing with respect to irradiation for thiolamines to be used to enhance the radiation sensitivity of cancer cells.

FY97 Publications, Presentations, and Other Accomplishments:

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Task Description:

The implementation of a space station, a lunar science base, deep space exploration, or high altitude commercial aircraft operations will result in substantially greater exposures to workers and the public from ionizing radiation than any prior activity. It is imperative that the associated health risk be made as low as reasonably achievable (ALARA) and be maintained within an acceptable level. The risk of injury of specific organs depends on the energy transfer processes from the radiation types present at the site to the local tissues. To ensure that acceptable risks are in fact achieved requires an adequate definition of several factors: the external space radiation environment, the modification of that environment by surrounding materials (including tissues), the understanding of the specific energy transfer processes to sensitive biological structures, and an adequate understanding of the biological response to this physical insult. Reducing risks requires control of the most biologically damaging components by adjusting the interaction with surrounding materials through selection and geometric arrangement. The purpose of this task is to develop computational procedures and corresponding databases for definition of the space radiation environment, interaction of that environment with appropriate materials through atomic and nuclear processes, transfer of energy to sensitive biological structures, and coupling to biological response models. The primary thrust of this task is the development of atomic and nuclear interaction databases and the transport these radiations through materials and the energy transfer processes to local tissues. The remaining activity is mainly through appropriate collaboration with other groups. The primary goal is to develop efficient computational procedures and corresponding databases which have been validated in laboratory experiments using specific ion beams and high resolution detectors for use in risk estimation for specific engineering designs. When coupled to environmental models, they can be further validated on specific flight platforms before use in future mission design. These methods when coupled to biological response models will provide the basis for maintaining risk at acceptable levels and in search of methods to keep risks ALARA in future NASA activity. A secondary goal of this task is to define dosimetric

data on specific ions for the interpretation of biological response data obtained in accelerator and space flight biological experiments. The preliminary codes and databases developed under this task are receiving wide acceptance in the engineering community (used in space exploration studies, in space station design, in the shuttle dosimetry program, in design of unmanned spacecraft, and were recently adopted by the Naval Research Laboratory for use in the Space Environment and Effects Project). Although great uncertainties remain in the methods and database, they are accepted as the best available and illustrates the potential impact of the current studies on space and aircraft technology. Near-term activity consists in re-evaluation of nuclear absorption and atomic cross section databases according to recent experimental data; re-evaluation of media modified two-body interaction amplitudes; development of nuclear cluster models for improved fragmentation dynamics (collaboration with workers at the new DOE Jefferson Research Laboratory); re-evaluation of the fragmentation database using the recent experiments of the BNL/AGS 1 AGeV Fe beam performed by J. Miller of LBL (199-45-16-12); development of a pion/kaon production database; development of higher order neutron propagators; determine effects of current biophysical models on shield characterization; examine dose rate effects in solar flare exposures (collaboration with Oak Ridge National Laboratory); examine effects of G1 and G2 blocking kinetics, cellular repair kinetics, and signal transduction (collaboration with Johns Hopkins Oncology Center); and develop analysis programs for space flight validation of environmental models, transport codes, and anatomical models (collaboration with JSC).

The purpose of this task is multifaceted with research objectives, evaluation of impact on shield design, and mission analysis activity in support of agency goals in deep space exploration and aeronautics development. The research objectives are the development of computational procedures and associated databases for the definition of the transmission characteristics of shield materials and biological tissues and validation of those procedures and databases by comparison with laboratory and flight experiments. The second objective is the evaluation of attenuation characteristics for specific biological response models as a guide to specification of shield effectiveness. The shield effectiveness depends on the nature of the biological response to the transmitted radiations and further research on and implementation of existing biological response models has been undertaken. The proceedings of the workshop on "Shielding Strategies for Human Space Exploration" are near completion and have had a major impact on the NAS study on "Radiation Hazards to Crews of Interplanetary Missions" and as input to the space shield materials research effort. Testing of shield materials at the BNL and Loma Linda facilities will be a new focus of the shielding program.

Research Progress. Much emphasis has been on database improvement and validation. The nuclear cross sections are still the major source of uncertainty and evaluation of our current needs was made by Cucinotta et al. Nuclear model and database improvements continue to be made in both the production of secondary particles and absorptive cross sections. Following the improved analysis of atomic stopping processes by Bichsel at the HIMAC, we re-evaluated our atomic database but found no need for modification. There has been no substantial change in our computational procedures although means of improving the low energy neutron transport section is under intensive investigation using classical multigroup methods (such as ANISIN) and development of new methods (Fn methods and a Greens function for the generalized multigroup equations). Monte Carlo methods even on modern computers are too slow to be useful in spacecraft shield analysis and design. Three orders of magnitude remain as the difference in computational time between analytical and Monte Carlo methods. Current activity involves the evaluation of the media modifications of the two nucleon amplitudes to improve the evaluation of cross sections in the intermediate and low energy region, the inclusion of pion production in the two body processes, addition of electromagnetic cascades to the transport code, and the development of nuclear cluster models to better estimate the reactive channel excitation energies.

Validation of the LaRC databases for fragmentation has been a focus of the LBL team using the 1.05 GeV/nucleon Fe beam at the BNL AGS accelerator and the NUCFRG2 and QMSFRG code are in reasonable agreement. In particular, QMSFRG showed the lowest average difference with the data than all other models considered (*Phys. Rev. C*56:388-397;1997). The greatest disagreement of QMSFRG with the data was for $\Delta Z < 4$ and was found to be in part attributable to a numerical truncation error which has since been corrected. Shield transmission studies have further improved our nuclear database. Validation in space flight is in progress using the TEPC as a radiation quality spectrometer. There has been a problem in the past using the TEPC as a quantitative instrument since the relation of spectral response for the many particle components was lacking.

J.L.Shinn of this project has, in collaboration with M. Xapsos of the Naval Research Lab, been improving the analysis of the space shuttle measurements. A model for the TEPC response modifications due to leakage of imparted energy to the detector wall, the short range of the low energy ions, and fluctuations in the energy loss of the passing ion has been used to reanalyze the shuttle data. Whereas estimates of the calculated LET spectra in the past have been different from the measurements by factors of 1.4 to 2.7, the new results using the TEPC response have greatly reduced the difference between theory and experiment. Further development to include energy transported into the detector by delta rays will further close the gap between measurements and theory. Experiments at the AGS by Colorado State University will further shed light on the accuracy of the model TEPC response functions allowing the TEPC to be used as a quantitative instrument for evaluation of transport code and environmental model accuracy.

Shielding Effectiveness. Shielding effectiveness depends on the particle types transmitted through the shield and the effectiveness of those particles to cause biological damage. It is well established that the track structure of individual ion types (LET, ion charge, ion energy) is a determining factor in biological response. It is found that dose equivalent for Galactic Cosmic Rays attenuates fairly well behind aluminum while the rate at which mouse cell cultures transform and the rate at which mice experience harderian gland tumors increase with increasing shield thickness and improved understanding of radiation risk behind shield materials is a critical issue for manned space exploration. Clearly improved risk models utilizing track structure dependent effects are needed in shield design specification.

Shield specification for protection from solar particle events is problematic since the size and spectral content of solar events are stochastic. We have addressed the shield design problem on the basis of defining a reasonable design event and then considered the possible harm to the astronaut by a possible larger event although their likelihood is small. The seriousness of these "accidental" exposures were examined using a human response model developed for the military. Issues on applicability of these models to microgravity environments are discussed. Shield penetration by the HZE particles of iron rich solar particle events can make a significant contribution to exposures in a spacesuit. Since the spectra of the HZE particles is very steep, an improved spacesuit model is required. A detailed model of the shuttle suit and the advanced Mark III suit are now in preparation for analysis.

Mission Analysis. Environmental data and shield attenuation studies were used to evaluate the exposures for the Human Lunar Return mission study and a possible Mars mission scenario. One result of this re-analysis is that the dose equivalent has increased by nearly a factor of two for typical spacecraft shielding as a result of improved nuclear databases. A rationale for protection from solar event exposures has also been developed in which design is for the largest event observed and the consequences of an even larger event can probably be handled by proper medical treatment. Such a larger event is very unlikely to occur and has not been observed in nearly four decades of observation. A number of improvements are now in progress for improved spacesuit, rover, habitat, and vehicle models. Furthermore, the transhab wall design is being tested at the BNL AGS facility as are other shield material concepts. A technology summary on High Speed Research radiation protection was also written for the management team for HSCT development.

The purpose of the present project is to improve our understanding of the role of materials in modifying the radiation fields of the broad class of ionizing radiation components in space for the purpose of modifying the radiation response of on-board biological and electronic systems. Potential benefits derive from applications to protection in the stray fields at particle accelerators, diagnostics for ion beam therapy, evaluation of RBE values for ion beam therapy applications in tumor reduction, improved estimates and mitigation of radiation health risks in high altitude commercial aircraft operations, and evaluation of single event upsets in modern aircraft designs and spacecraft designs.

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Molecular Analysis of HZE Damage in Transgenic Mice

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Task Description:

Although there is evidence, both *in vivo* and *in vitro*, for the mutagenic and clastogenic potential of HZE particles, there are few studies on the molecular mechanisms of this damage in animals or humans. This is primarily because *in vivo* mutation assays could be performed only with difficulty and in very few tissues. The development of transgenic mouse mutation assays now allows the rapid detection, quantification, and molecular analysis of mutations induced in any tissue of the animal. We have recently shown that it is possible with these assays to detect and characterize mutations induced in different tissues by both gamma and alpha particles. We are using a transgenic mouse system that has been constructed so that every cell of the animal contains multiple copies of an integrated target gene. The use of this system will allow us: 1) to recover and quantify radiation-induced mutations readily; 2) to determine the nature of these mutations by restriction fragment length polymorphisms (RFLP); 3) to investigate the molecular mechanisms of mutation induction by DNA sequence analysis of recovered mutants; and 4) to assess whether there are tissue-specific mutagenic mechanisms induced by HZE radiation, especially in nondividing tissues and germ and stem cells. The advantage of the transgenic mouse is that, in parallel with experiments using the integrated target gene, it will be possible to assess mutagenicity at another locus (hypoxanthine guanine phosphoribosyl transferase, *hprt*) and to use the micronucleus assay and chromosome painting to assess the frequency of cytogenetic damage induced in the bone marrow and peripheral blood erythrocytes of these mice. These last three end points can also be assayed in humans, using blood samples collected by venipuncture. This allows mutagenic and clastogenic endpoints to be correlated between the two species. We also propose using transgenic mice that have been crossed with mice which have either one or both copies of the *p53* tumor suppressor gene inactivated. This gene is involved in responses to ionizing radiation, and the use of these mice should be informative for the mechanisms of radiation action. Mice that are hemizygous at the *p53* locus should serve as models for individuals in the human population who may be carriers of recessive genes that are predisposed to cancer.

We are using the C57lacZ transgenic mouse line, which contains multiple copies of the bacterial reported lacZ gene integrated into every cell of the animal, to measure the heavy particle radiation-induced mutation frequency *in vivo*. We are interested in examining the contribution of genetic background in the response of animals to

radiation-induced genetic damage. We have therefore cross-bred the CBA mouse line with our C57lacZ line such that the lacZ reporter gene is also integrated into the genome of these animals with a genetic background that is known to be prone to genomic instability. (In addition, we have also crossed the C57lacZ mice with those that have a targeted neo insertion into the p53 gene and thus are nullizygous for p53 protein expression (supplied by Jackson Labs, Bar Harbour, ME)). The mouse lines we have established, therefore, all contain the lacZ gene integrated into the genome, but in addition, are either hemizygous or nullizygous to p53.

We exposed the animals to 1 Gy of 1 GeV/amu iron ions and analyzed the induced mutation frequency in different tissues at sequential times following heavy-ion irradiation. In the same animals, we also measured the induction of micronuclei in peripheral blood (work performed in collaboration with D. Toreous, Litron Laboratories, NY) and the mutation induction at the endogenous hprt locus (work performed in collaboration with Dr. V.E. Walker, Waldsworth Center, NY).

At 72 hours after 1 Gy of Iron ions, we saw a 2.3-2.6 fold increase in the amount of micronuclei in the circulating polychromatic erythroblasts (PCE) compared to the unirradiated controls in the C57lacZ, CBA lacZ, and the p53 hemizygous lacZ animals. In the p53 nullizygous lacZ animals, we saw a greater (4.3-fold) induction. The dynamics of removal of micronuclei in PCEs appear different for animals with different genetic backgrounds. In the C57lacZ and p53^{+/-}-lacZ animals, the level of micronuclei induction was reduced to control levels in less than 9 days post irradiation. In the CBA lacZ animals, the level of micronuclei was still elevated (1.6-fold greater than the controls) 9 days post irradiation, returning to control levels 4 weeks post irradiation. In the p53^{-/-}-lacZ animals, the level of micronuclei in the PCEs remained elevated (1.5-fold greater than the controls) 4 weeks post irradiation.

Mutant frequencies (MF) in the endogenous hprt locus were measured in T-lymphocytes 4 and 8 wks after radiation. It appears that the mutation frequencies are elevated at 4 wks post irradiation (11-fold) and decrease to about 2 fold 8 wks post irradiation in the C57lacZ animals. Consistent with our previous novel observation that loss of p53 function can induce changes in mutation frequencies, we measured a 20-fold increase in MF in the p53^{+/-} animals and a 40 fold increase in the p53^{-/-} animals 4 weeks after radiation. These increased levels of MF decreased with time, such that by 8 weeks post irradiation, the MF for p53^{+/-} is about 7-fold above control, and the MF for the p53^{-/-} is about 12-fold above the control.

We conclude from our findings thus far that exposure to iron ions resulted in a dramatic immediate response in (1) the hematopoietic system, as indicated by a large induction in micronuclei in the circulating PCEs and (2) T-lymphocytes, as indicated by the high mutation frequency of the endogenous hprt locus. The recovery from genetic damage, as measured in these two endpoints, is dependent on the genetic make-up of the individual animals.

The results of our recent work raise several questions. First, what is the mechanism for sensitivity of p53^{+/-} hemizygotes to radiation-induced hprt mutations? Do the lymphocytes containing these mutations have an elevated rate for loss of the remaining p53 gene? Or is there truly a gene dosage effect? How relevant are these results to humans who may be carriers of only a single copy of the wild-type p53 gene? Additionally, it is interesting that we do not see a significant increase in sensitivity among p53^{+/-} hemizygotes to micronucleus induction compared to the controls. Is this due to the endpoint measured (clastogenesis vs. gene mutation) or to the type of tissue examined (erythrocytes vs. lymphocytes)?

In future studies, we will attempt to answer some of these questions. By analyzing the individual selected hprt mutant clones, we will be able to determine the p53 status of these cells. We would also like to investigate measuring micronuclei in lymphocytes to determine whether the enhanced hprt mutation response in p53^{+/-} hemizygotes is endpoint specific or a tissue specific response. We will continue with the mutation analyses of the target lacZ transgene in a wide variety of tissues such as spleen, liver, brain, and germ cells.

This research is directed towards an understanding at the molecular level of the effects in humans of exposure to ionizing radiation. Although it is known that such exposure can cause life-shortening carcinogenesis,

chromosome abnormalities, neurological damage, tissue damage, and cataractogenesis, the underlying molecular mechanisms for the induction and processing of this damage have not been well-characterized. An understanding of the basic molecular processes that are involved in the resolution of ionizing-radiation induced damage will enable greater accuracy in predicting the magnitude and nature of the response to ionizing radiation exposure in humans. It can also provide information on fundamental cellular processes such as the damage induction and response pathways, cell cycle controls, tissue-specific mutagenic mechanisms, and the induction of genomic instability. The assays that we are developing in these studies have the potential to provide a direct correlation between the damage induced by ionizing radiation, both on Earth and in space, in experimental animals and in exposed human populations. With a greater understanding of the fundamental mechanisms that underlie responses to ionizing-radiation exposure, and with the development of endpoints that can be assayed in both the human and experimental animal models, it should be possible to improve risk estimates for individuals who are exposed to ionizing radiation, whether it is environmental, man-made, or cosmic.

Neoplastic Cell Transformation With Protons and HZE

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Task Description:

The energetic electrons, protons, and heavy ions that constitute space radiation are hazardous to human health. High-LET heavy ions are particularly effective in causing various biological effects, including cell inactivation, mutation, and cancer. Among these biological effects, the induction of neoplasm is the most important late effect to be considered in radiation risk assessment because astronauts usually are chronically exposed to low doses. During a long-term space flight, such as a mission to Mars, astronauts will be exposed to considerable amounts of galactic cosmic rays (GCR). To ensure proper protection of astronauts and the success of a long-term mission, a better understanding of the carcinogenic effects of energetic protons and heavy ions is most essential. The major objectives of this proposal are to quantitatively measure the oncogenic effects of energetic protons with tissue-equivalent material shielding, to determine the relative biological effectiveness (RBE) of high-energy heavy ions, to gain a better understanding of the kinetics of repair of oncogenic damage, to examine the carcinogenic effect of gamma rays at very low dose rates, and to characterize the changes of growth properties and karyotype of radiation-transformed cells. We will show: 1) that high-energy heavy ions (>1GeV/u) are effective in causing neoplastic transformation of mammalian cells and in producing irreparable oncogenic lesions; 2) that the effectiveness of protons in causing cell transformation will be altered by tissue-equivalent material shielding; and 3) that the dose rate reduction factor for very low dose rate gamma rays can be more than three in confluent (G1) cells. To accomplish these objectives, we will conduct proton experiments using the synchrotron at the Loma Linda Medical Center in California during the first and second year, high-energy heavy-ion studies at AGS of Brookhaven National Laboratory in the second and third year, and low-dose-rate experiments with the gamma-ray source at NASA Johnson Space Center when proton and ion beams are not available. Experimental results so obtained should significantly increase our knowledge of the oncogenic effects of space radiation and should help to reduce the large uncertainty presently existing in radiation risk assessment.

During this reporting period, the experiments done were mainly on collecting quantitative and qualitative information on cell killing, chromosomal aberrations, and neoplastic cell transformation by charged particles with and without shielding using preliminary data obtained last year to design these experiments. Molecular studies with human cells transformed by heavy ions were continued.

We have conducted experiments with 250 MeV protons at Loma Linda University Medical Center for survival and chromosomal aberrations determination. The kinetics of chromatin damage repair, formation of various types of chromosomal aberrations, including translocation, dicentrics, etc., in G₀/G₁ human cells were determined with fluorescence *in situ* hybridization (FISH) and premature chromosomal condensation (PCC) techniques. Preliminary results showed that the total number of fragments decreased with incubation time, suggesting rejoining of chromatin breaks. The translocation and dicentrics, however, increased with time and reached a plateau about 10 hr post irradiation. For survival and cytogenetic damages, the RBE value was about 1.0.

A better understanding of both the acute and the late effects of high-LET charged particles is essential for assessing radiation risk to astronauts for long-term space exploration missions. We conducted cell experiments with 1 GeV/u iron particles accelerated at AGS of Brookhaven National Laboratory in 1996 and have obtained some preliminary results on lethal and cytogenetic effects of these particles in mammalian cells. Cell killing can lead to functional alterations in tissues and cytogenetic damages can cause mutation and oncogenic transformation. We irradiated normal human lymphocytes and mouse embryonic fibroblasts (C3HIOT1/2) *in vitro* to determine the dose-response relationships for chromosomal aberrations and cell killing, respectively. We used FISH techniques for chromosomal aberrations analysis and FISH and PCC to measure the kinetics of rejoining and misrejoining of chromatin breaks. Preliminary results showed that the dose-response relationship is linear and about the same for initial induction of PCC fragments in human lymphocytes exposed to gamma rays, protons or iron particles. The kinetics of rejoining of PCC fragments, however, was different for different types of radiation. Less than 10% of initial number of fragments, for example, could be observed in cells exposed to protons or gamma rays after 10 hr incubation at 37°C. About 50% of PCC fragments, on the contrary, were found in cells exposed to iron particles at 10 hr post irradiation. While the total amount of PCC fragments decreased with incubation time, the frequency of complex exchanges and total exchanges per cell increased exponentially and reached a maximum at about 10 hr. Interestingly, there was no significant difference in kinetics for exchange formation induced by protons, iron particles, or gamma rays. Our results also showed that the RBE for different PCC chromatin aberrations was not the same—highest for reciprocal exchanges and lowest for complex exchanges. Our survival studies indicated an exponential dose-response curve for mouse embryonic fibroblasts (C3HIOT1/2) exposed to iron particles with 0- or 5-cm tissue equivalent material shielding. The survival curve for IOT1/2 cells, however, showed an increase of survival and a small shoulder when the plating was delayed for about two days. Furthermore, the cells with 5-cm tissue equivalent material shielding showed significantly higher survival rate than that without shielding, suggesting fragmented iron particles can be less effective in causing cellular damages. These findings are in consistent with our hypotheses and suggest further studies.

Molecular analysis of cancer genes in human mammary epithelial cells transformed by heavy ions was continued. The expression of both p53 and Rb genes was determined and no difference was found from that in control cells. However, experimental results from cell fusion studies done in our laboratory indicated that the transforming gene(s) is recessive. Recently we have conducted experiments with 1 GeV/u iron particles and found that RBE for complex exchanges is higher than that for other types of aberrations. The relationship between complex exchanges and the inactivation of tumor suppressor genes remains to be determined.

In addition to above studies, we recently performed cell experiments with carbon and neon ions generated at the Heavy-Ion-Medical Accelerator in collaboration with scientists at the National Institute of Radiological Sciences (NIRS) in Chiba, Japan. Irradiated samples are under analysis.

This research work seeks to understand the carcinogenic effects of low- and high-LET radiation in mammalian cells. Radiation is part of our environment and can cause genetic alterations and cancers in humans. On the other hand, with proper control, ionizing radiation, including x-rays, g-rays, neutrons, and charged particles, can be useful for treating various human diseases. In fact, cancer radiotherapy with protons and charged particles is either in development or in practice in USA, Asia, and Europe. Our research studies on the effectiveness of low-LET radiation at low dose rates in causing oncogenic cell transformation will add quantitative information to the existing data for assessing radiation risk to ground radiation workers. Data from our high-LET radiation

studies will be valuable for understanding the health effects of neutrons to radiation workers of nuclear reactors and for estimating the cancer risk of radon gas to the general public. The shielding studies with protons and carbon ions provide useful information for making treatment plans since tissue-equivalent materials and the human body are part of the necessary shielding during radiotherapy. The cell fusion experiment, cancer genes studies, and the analyses of chromosomal aberrations in non-transformed and tumorigenic cells shed light on the basic mechanism(s) of carcinogenesis by radiation.

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Wu, H., Durante, M., George, K., and Yang, T.C. "Induction of chromosome aberrations in human cells by charged particles." *Radiat. Res.*, 148, S102-S107 (1997).

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A Model for Down Regulation of Erythropoiesis in Space

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Funding:

UPN/Project Identification: 199-14-17-16

Solicitation: 96-OLMSA-01

Initial Funding Date: 1996

Expiration: 1996

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Upon entry into microgravity astronauts suddenly have too many red blood cells (RBCs), an event rarely experienced on Earth. Studies on six space shuttle crew members (SLS 1 and SLS 2) showed that newly produced RBCs or cells scheduled for release from bone marrow were selectively destroyed, accounting for the decrease in red blood cell mass (RBCM) that has been found after spaceflight. These observations provide the first evidence that RBCM in circumstances of RBC excess is downsized by destruction of newly produced cells. High altitude acclimatized persons transported to sea level have a RBCM in excess to that optimal for the environment of sea level. This mirrors the changes we have observed in astronauts, thus at sea level newly produced RBCs should be destroyed to reduce RBCM.

We propose to study altitude acclimatized persons before and after transport to sea level. The RBCM and excretion of fecal stercobilin will be followed serially. The latter reflects the rate of total heme catabolism. Heme precursors, 15N- and 13C-glycine will be given and fecal stercobilin enrichment will be used to quantitate destruction of newly formed RBCs. 15N- and 13C-enrichment of blood heme and RBCs labeled with 51Cr will be used to follow the survival rates for RBCs of different ages. The fall in serum erythropoietin levels when there is a RBC excess may allow for the destruction of newly formed RBCs. To test this hypothesis, half of the subjects (7) will receive injections of erythropoietin while at sea level to determine if destruction of RBCs will be blunted. This study could confirm or refute our hypothesis for control of the size of the RBCM that resulted from our spaceflight research. These studies will provide insight into mechanisms of anemia and pharmacology of erythropoietin.

In April 1997, we conducted the experiments described above. We studied nine subjects who were long-time residents of the city of Cerro de Pasco, Peru (altitude: 14,500 feet). As a consequence of the hypoxia at this level, all of these individuals had increased red blood cell mass, hemoglobin, and hematocrit. They also had elevated levels of erythropoietin. We gave them C-13 glycine and collected stool specimens. The stool

specimens were collected for the purpose of quantitating stercobilin excretion and also the enrichment of the stercobilin with C-13 which would result from destruction of newly synthesized hemoglobin. After observation for 2 weeks in Cerro de Pasco, these nine individuals were transported by a bus to Lima, Peru (which is at sea level) where they remained for the following two weeks.

We studied the individuals every other day; measuring their hemoglobin and hematocrit, collecting stool specimens and blood samples for analysis of erythropoietin, transferrin receptor, and serum ferritin. Three of these individuals were given erythropoietin injections to see if this hormone was responsible for prevented their de-adaptation to sea level. We determined that all individuals who received no erythropoietin had a decrease in red cell mass and hematocrit with decreases up to 15% occurring over an eleven day period. All individuals who had a decrease in their erythropoietin except of course for those who were receiving erythropoietin injections. All individuals who received no erythropoietin had progressive increase in their serum ferritin reflecting an increase in iron stores resultant from the decrease in red cell mass. Erythropoietin administration completely blocked the changes in the serum ferritin, i.e., there was no increase in iron storage in those people who received erythropoietin shots and similarly there was no decrease in red cell mass or hemoglobin or hematocrit in those people who received erythropoietin.

Following descent to sea level in Lima, the individuals had an increase in the enrichment of the fecal stercobilin with C-13 and 15 indicating that there was selective destruction of newly produced red blood cells.

These studies indicate that individuals with red blood cells in excess of that optimal for the environment have destruction of selected red cells, particularly recently produced red cells. This decrease or selective destruction of cells is mediated by a decrease in erythropoietin; at least the decrease in selective destruction of cells is prevented by administration of erythropoietin even when the individual is in an environment in which the optimal number of circulating red cells is decreased.

These studies support our previous observations in space flight that man adapts to circumstance in which red cells are in excess by selective destruction of red cells, particularly newly produced red cells. The destruction occurs as a consequence of a decrease in erythropoietin which occurs both in astronauts and in individuals who descend from altitude to sea level.

These studies suggest that individuals who suddenly have an excess of red cells relative to the requirements for their environment have a rapid decrease in the number of circulating cells and thus those individuals who come from high altitude to sea level rapidly de-adapt to their high altitude environment. Our studies clearly demonstrate that this rapid de-adaptation to the high altitude environment is likely to cause individuals who have need for aerobic activities when returning to high altitude to be significantly de-adapted even after a period at sealevel of a few days. This de-adaptation appears to be preventable by the administration of erythropoietin.

As previously indicated by these investigators, the rapid changes resultant from a decrease in erythropoietin are important with regard to the treatment of the anemia of renal disease, particularly with regard to the schedules for administration of erythropoietin. Erythropoietin given intravenously or even subcutaneously has a half-life of only a few hours and if the erythropoietin is given at intervals of 2-3 days as is generally the case by many physicians treating the anemia of renal disease neocytolysis or destruction of newly synthesized red cells is likely to occur as a consequence of decrease in erythropoietin below a threshold level. This research will provide a new understanding of basic biologic principles as we understand the mechanism by which newly produced red cells are selectively captured and destroyed.

These studies clearly show the value of observations while in space. They provide insights into important biologic mechanisms that would not have been appreciated had astronauts not been studied in space.

This research should allow us to better understand how individuals adapt to high altitude and how their de-adaptation to altitude can be prevented when they must make short sojourns to a lower altitude. The processes that we have identified should be useful in allowing individuals who must work at high altitude to

remain adapted to that environment. And thus a very large portion of our population can potentially benefit from the results of these studies.

FY97 Publications, Presentations, and Other Accomplishments:

Alfrey, C.P., Rice, L., Udden, M.M., and Driscoll, T.B. "Neocytolysis: Physiological down-regulator of red-cell mass." *Lancet*, 349-1389-90 (1997).

Intestinal Adaptation in Microgravity Drug and Nutrient Adsorption

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Funding:

UPN/Project Identification: 199-18-17-20
Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$99,386

Solicitation: 95-OLMSA-01
Expiration: 1997
Post-Doctoral Associates: 1

Task Description:

The physiologic changes which occur in microgravity environments can have a significant effect on drug and nutrient absorption and metabolism. The long-term goal of this research project is to quantitate the alterations of gastric emptying and intestinal transit as well as drug absorption and metabolism in a microgravity environment.

The primary objective during the past year has been to analyze the results of pellet gastric emptying test to determine the influence of size, density, and gravity on particle emptying from the stomach. Dosage forms prepared by Pharmacia & Upjohn Company have been evaluated in a human study and the results indicate that small particles always empty ahead of large particles in the fed-state and that the salivary levels correlate extremely well with plasma levels for both caffeine and acetaminophen, the test drugs in the two difference size pellets administered as part of the test system. Results of this project have been written up and are included in an abstract as well as in a thesis (Julie Rhie, PhD thesis, University of Michigan) and in a draft manuscript. A second study has been initiated to determine the effect of a normal meal on gastric emptying discrimination of this particle-based pellet gastric emptying test as well as to establish a control value showing similar gastric emptying of same size particles of acetaminophen and caffeine. This will further validate the pellet gastric emptying test system for use in a microgravity environment based on salivary sampling. It has important implications for both drug absorption as well as nutrient absorption and gastrointestinal adaptation in a microgravity environment.

The tasks for 1997 included the following: detailed analysis of the pharmacokinetic parameters associated with gastric emptying of pellets of caffeine and acetaminophen of different particles sizes; completion of the statistical analysis to confirm the size discriminating capability of the gastric emptying of particles in the fed-state; and to establish a protocol for further studies with food and particles of similar size to establish that different times of appearance of acetaminophen and caffeine in the plasma is due to the size differences in the particles used in the test system rather than the absorption rate differences, which is expected to be negligible for both of these well-absorbed compounds. The protocol for the validation of the gastric emptying test under normal food test meal as well as further validation of the size discrimination capability of this test has been approved and will be completed soon.

This project will evaluate a new, non-invasive test to assess gastric emptying, the PGE test. This test could be used to assess gastric emptying on Earth as well as in space. Alterations in gastric emptying occur in many disease states, including diabetes mellitus, AIDS, critical illnesses, viral infections, and gastroesophageal reflux disease. Current methods to assess gastric emptying include manometry and or scintigraphy. These methods are invasive, cumbersome, associated with risks, and costly. The new PGE test would allow for easy, serial, non-invasive assessment of gastric emptying.

FY97 Publications, Presentations, and Other Accomplishments:

Rhie, J.K. The pellet gastric emptying (PGE) test: Development of a non-invasive method to assess gastric emptying. (Dissertation) The University of Michigan, College of Pharmacy, Ann Arbor, MI (December, 1996).

Rhie, J.K., Hayashi, Y., Welage, L.S., Wald, R.J., Barnett, J.L., Amidon, G.E., Putcha, L., and Amidon, G.L. "Size differentiated gastric emptying assessment in humans with the non-invasive pellet gastric emptying (PGE) test." *Pharm. Res.*, 13(S), S-283 (1996).

Adaptive Visual-Vestibular Mechanisms and Gravity

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Funding:

UPN/Project Identification: 199-16-17-13

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$64,303

Task Description:

Throughout the history of the manned space flight program, microgravity has produced vestibular-related symptoms that result in personal discomfort and a loss in crew performance. The symptoms subside within several days of microgravity exposure, suggesting that interactive visual-vestibular mechanisms may be responsible for the initiation of symptoms and the subsequent adaptation. In order to better understand the nature of visual-vestibular adaptation mechanisms and their effects upon motor function, the processes underlying neural plasticity and adaptation under conflicting sensory conditions must be established. This project will provide experimental and theoretical data regarding integration of multisensory inputs and adaptive changes in gravity-sensitive central mechanisms during orientation and movement in space.

The vestibulo-ocular reflex (VOR) generates compensatory eye movements in response to changes in head position, velocity, or acceleration in space and has been shown to be affected by space flight conditions. This project examines the adaptive changes in the VOR before, during, and following exposure to an altered ("tilted") visual environment in rhesus monkeys. The rotation of the visual world will be generated either through optical devices mounted on both eyes (long-term adaptation) or through simultaneous vestibular and optokinetic stimulation about two orthogonal head axes (short-term adaptation). Eye movements will be monitored in three-dimensions in order to determine the spatio-temporal organization of the vestibulo-ocular reflex following adaptation to an optical tilt. During exposure to a visually tilted environment, the Earth-vertical direction signaled by the otolith receptors and the vertical cues provided by the visual inputs are no longer in register. The main goal of the proposed project is to investigate whether and how, under these conditions, coding of Earth-vertical is reorganized and reinterpreted such that there will no longer be sensory conflict between the visual and vestibular signals. It is hypothesized that, after adaptation to a tilted visual environment, the vestibular system still monitors Earth-vertical via the otolith receptors; however, this spatial vertical is no longer aligned with the gravitational force, but rather shifted in the direction of tilt of the visual world. The proposed project will provide information about integration and fusion of multisensory inputs and adaptive changes in gravity-sensitive central mechanisms. Such information about adaptive changes in the central vestibular system during conflicting, non-complementary visual-vestibular interactions are important for a better understanding of similar adaptive changes that occur in microgravity during space flight.

Since the beginning of the funded period, the PI's laboratory is fully functional and equipped with a unique three-dimensional motion delivery system that can provide any combination of linear and angular motion stimuli

in three-dimensions. Experimental animals have been implanted for experiments and trained for three-dimensional eye movement recordings. Experiments associated directly with the Specific Aims of the research are currently in progress and several interesting results are being obtained during preliminary analyses of the data (see below). In addition, on-going work on topics directly relevant to NASA interests about the role of gravity-sensitive responses on the vestibulo-ocular reflex have been completed and published in peer-reviewed journals (see Publication list).

A main goal of this project is to study the ability of the vestibular system (semicircular canals and otoliths) to adapt to altered sensory conditions as registered by retinal slip associated with non-complementary visual stimulation. In the laboratory, this is easily achieved by rotating the animals about an axis, i.e., yaw, whereas an optokinetic stimulus is being presented about a different axis, i.e., pitch. When the axis of rotation is maintained Earth-vertical throughout the motion, only semicircular canal receptors are dynamically stimulated. When the axis of rotation is Earth-horizontal, both semicircular canals and otolith receptors are stimulated. Following repeated exposure to these conditions, the vestibular system "learns" to generate a pitch (vertical) eye movement during subsequent yaw rotations in complete darkness.

Preliminary analyses have shown several interesting results: (a) When only the semicircular canal system is asked to adapt, the adaptive properties are uniform for all directions of motion and optic flow stimulation; (b) When also the otolith system is activated, the adaptive properties of the vestibulo-ocular system change: Certain combinations of vestibular/visual directions are easier coupled than others; (c) Whenever the otolith system is dynamically activated during adaptation to the non-complementary vestibular/visual conditions, the extent and speed of adaptation is augmented, particularly during low frequency motion; (d) The increased contribution of the otolith system to vestibular/visual adaptive properties is even more conspicuous in the fact that it is able to adaptively "learn" to generate an orthogonal eye movement at a different frequency from that of the associated head and body motion. When, for example, the accompanying visual stimulation occurs at a frequency that is double from that of head rotation, the orthogonal component that the system learns to generate during subsequently motion in complete darkness is at this double frequency.

These preliminary results are very important for our understanding of visual/vestibular interactions during normal and non-complementary sensory conditions. Continuing experiments and more detailed analyses will further increase our understanding of these properties. In normal behavior, as well as in the extraordinary challenges of altered gravity, spatial orientation and motor coordination is an intriguing task due to its complexity and the need for integration of movement information from several sensory modalities including the vestibular, the visual, and somatosensory systems. Even for a single system, for example the vestibular system, the otolith and semicircular canals provide distinct information which must be centrally fused, integrated, and re-interpreted. Our efforts have provided interesting experimental results and theoretical insights into the process of extracting motion information from a combination of otolith and semicircular canal information.

The research funded by this NASA grant aims to understand basic mechanisms underlying the normal organization and coordination of otolith, semicircular canal, and visual signals. By comparing the normal with the adapted visual/vestibular mechanisms underlying eye movement control and spatial orientation, these studies aim at improving our understanding of such multisensory integration questions in normal and disease states not only on Earth, but also in space where altered gravity/visual interactions provide a demanding challenge on our cognitive and motor functions. Results of these studies will be important in understanding the process of sensory adaptation to altered visual/vestibular conditions experienced during space travel and upon return to the Earth's gravitational environment.

FY97 Publications, Presentations, and Other Accomplishments:

Angelaki, D.E. and Hess, B.J.M. "Adaptive modification of primate vestibulo-ocular reflex to altered peripheral vestibular inputs. II. Spatiotemporal properties of the adapted slow phase eye velocity." *J. Neurophysiol.*, 76, 2954-2971 (1996).

Angelaki, D.E. and Hess, B.J.M. "Visually-induced adaptation in three-dimensional organization of primate vestibulo-ocular reflex." *J. Neurophysiol.* (In Press).

Angelaki, D.E. and Hess, B.J.M. "Adaptation of vestibulo-ocular reflex after selective semicircular canal plugging." *Soc. Neurosci. Abstr.*, Washington DC (November 16 - 21, 1996).

Angelaki, D.E., Dieterich, M., Zink, R., Suzuki, J.-I. and Hess, B.J.M. "Adaptive changes in spatio-temporal properties of 3-dimensional vestibulo-ocular reflex after selective semicircular canal plugging" in "Three-Dimensional Kinematics of Eye-, Head- and Limb Movements." Edited by: Fetter, M., Misslisch, H., and Tweed, D. Harwood Academic Publishers, Amsterdam (In Press).

Angelaki, D.E., Hess, B.J.M., Arai, Y., and Suzuki, I.-J. "Adaptive modification of primate vestibulo-ocular reflex to altered peripheral vestibular inputs. I. Frequency-specific recovery of the horizontal VOR after inactivation of the lateral semicircular canals." *J. Neurophysiol.*, 76, 2941-2953 (1996).

Angelaki, D.E., McHenry, M.Q., and Hess, B.J.M. "Primate vestibulo-ocular responses during linear motion." 9th European Conference on Eye Movements, Ulm, Germany (September 23 - 26, 1997).

Dickman, J.D., Beyer, M., Newlands, S.D., McHenry, M.Q., Hess, B.J.M., and Angelaki, D.E. "Linear vestibulo-ocular responses in pigeons." 9th European Conference on Eye Movements, Ulm, Germany (September 23 - 26, 1997).

Hess, B.J.M. and Angelaki D.E. "Dynamic control of primary eye position as a function of head orientation relative to gravity" in "Three-Dimensional Kinematics of Eye-, Head-, and Limb Movements." Edited by: Fetter, M., Misslisch, H., and Tweed, D. Harwood Academic Publishers, Amsterdam (In Press).

Hess, B.J.M. and Angelaki, D.E. "Kinematic principles of primate rotational vestibulo-ocular reflex. I. Spatial organization of fast phase velocity axes." *J. Neurophysiol.* (In Press).

Hess, B.J.M. and Angelaki, D.E. "Kinematic principles of primate rotational vestibulo-ocular reflex. II. Gravity-dependent modulation of primary eye position." *J. Neurophysiol.* (In Press).

Hess, B.J.M. and Angelaki, D.E. "Gravity-induced dynamic modulation of vestibulo-oculomotor coordinates." 9th European Conference on Eye Movements, Ulm, Germany (September 23 - 26, 1997).

Si, X., Angelaki, D.E., and Dickman, J.D. "Functional characterization of pigeon primary otolith afferents to linear acceleration." *Soc. Neurosci. Abstr.*, Washington DC (November 16 - 21, 1996).

Adrenoreceptor Hypersensitivity in Models of Weightlessness

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Funding:

UPN/Project Identification: 199-08-17-61

Solicitation: not available

Initial Funding Date: 1993

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 3

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Our central hypothesis is that alterations in autonomic function occur because of exposure to microgravity, and that these alterations are likely responsible for many of the physiological responses to weightlessness. Our overall aim remains to understand the alterations in the autonomic nervous system observed in models of weightlessness. Two models are being studied: bedrest deconditioning, and patients with orthostatic intolerance. In the current funding year we have established state of the art techniques in our laboratory to determine the effects of bedrest on sympathetic function. Our preliminary studies also provide evidence of hyper-responsiveness of adrenergic agonists in patients with orthostatic intolerance, and alterations in regional norepinephrine spillover. These patients have a clinical picture similar to that observed in astronauts upon return to 1-G.

In the past year we have expanded our observations in clinical conditions characterized by idiopathic orthostatic intolerance (OI). The purpose of these studies was to determine potential pathophysiological mechanisms of OI. Hyperadrenergic orthostatic intolerance is the most common form of dysautonomia disabling young otherwise healthy individuals. It is also seen in the initial stage of diabetic autonomic neuropathy. This condition is identical to that observed in astronauts as they return to Earth. In FY97, we have used regional norepinephrine spillover to determine if these patients have regional differences in norepinephrine spillover. Our preliminary observations suggest that there is impaired norepinephrine release in the lower limbs in these patients, particularly during stimuli that would produce sympathetic activation analogous to standing. This impairment in sympathetic activation in lower extremities may explain the difficulty these patients have in maintaining upright blood pressure and may underlie their orthostatic intolerance. These initial results underscore the importance of the studies to be performed in the Neurolab shuttle flight, which are design to test this hypothesis in astronauts.

Orthostatic intolerance is a significant cause of disability in otherwise normal young people. Even though it is the most common disturbance of the autonomic nervous system, its pathophysiology is incompletely understood. There is, therefore, no satisfactory treatment for this condition. Our recent studies have also characterized an orthostatic intolerance similar to that of astronauts also occurs in some patients with diabetes

mellitus. It is hoped that understanding the mechanisms of orthostatic intolerance will lead to improved treatment of this disease.

FY97 Publications, Presentations, and Other Accomplishments:

Biaggioni, I. "Erythropoietin in autonomic failure" in "Primer on Autonomic Function." Edited by: Robertson, D., Low, P.A., and Polinsky, R.J. San Diego: Academic Press, pp 332-334 (1996).

Biaggioni, I., Costa, F., and Kaufmann, H. "Vestibular influences on autonomic cardiovascular control in humans." *J. Vestib. Res.*, 8(1), 1-7 (1997).

Costa, F.A., Davis, S.N., and Biaggioni, I. "Exercise, but not ischemia, increases muscle interstitial adenosine in humans." *Med. Sci. Sports Exercise*, 29, S182 (1997).

Costa, F.C. and Biaggioni, I. "Biochemical evidence that adenosine mediates the exercise pressor reflex." *Clin. Autonom. Res.*, 7(5), 261 (1997).

Costa, F.C., Davis, S.N., and Biaggioni, I. "Interstitial adenosine concentrations correlate with sympathetic activation induced by ischemic exercise in humans." *Circulation*, 96(8), I-364 (1997).

Jacob, G., Robertson, D., Mosqueda-Garcia, R., Ertl, A., Robertson, R.M., and Biaggioni, I. "Hypovolemia in syncope and orthostatic intolerance. Role of the renin-angiotensin system." *Am. J. Med.*, 103, 128-133 (1997).

Jacob, G., Robertson, R.M., Costa, F., Wathen, M.S., Stein, M., Black, B., Biaggioni, I., and Robertson, D. "The local norepinephrine spillover in arms and legs of patients with orthostatic intolerance: Evidence of partial dysautonomia." *Clin. Autonom. Res.*, 7(5), 235 (1997).

Jacob, G., Shannon, J.R., Black, B., Biaggioni, I., Mosqueda-Garcia, R., Robertson, R.M., and Robertson, D. "Effects of volume loading and pressor agents in idiopathic orthostatic tachycardia." *Circulation*, 96(2), 575-580 (1997).

Jordan, J., Shannon, J.R., Biaggioni, I., Robertson, R.M., Norman, R., Black, B., and Robertson, D. "Contrasting actions of pressor agents in severe autonomic failure." *Clin. Autonom. Res.*, 7(5), 261 (1997).

Jordan, J., Shannon, J.R., Black, B., Costa, F., Ertl, A.C., Furlan, R., Biaggioni, I., and Robertson, D. "Malignant vagotonia due to selective baroreflex failure." *Hypertension*, 30, 1072-1077 (1997).

Jordan, J., Shannon, J.R., Black, B., Costa, F., Furlan, R., Biaggioni, I., and Robertson, D. "Malignant vagotonia due to selective baroreflex failure." *Clin. Autonom. Res.*, 7(5), 247 (1997).

Jordan, J., Shannon, J.R., Grogan, E., Biaggioni, I., Black, B.K., Robertson, R.M., and Robertson, D. "Pressor effects of oral water in primary autonomic failure." *Circulation*, 96(8), I-740 (1997).

Robertson, D., Jacobs, G., Ertl, A., Shannon, J., Mosqueda-Garcia, R., Robertson, R.M., and Biaggioni, I. "Clinical models of cardiovascular regulation after weightlessness." *Med. Sci. Sports Exercise*, 28, S80-S84 (1996).

Shannon, J., Jordan, J., Costa, F., Robertson, R.M., and Biaggioni, I. "The hypertension of autonomic failure and its treatment." *Hypertension*, 30, 1062-1067 (1997).

Shannon, J.R., Jordan, J., and Biaggioni, I. "Pupillometry in the assessment of autonomic function, and adrenergic and cholinergic sensitivity." *Clin. Autonom. Res.*, 7(5), 243 (1997).

*Neuronal Vulnerability and Informatics in Human Disease [Human Brain Project]***Principal Investigator:**

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 Warren Young, Ph.D.; The Scripps Research Institute

Funding:

UPN/Project Identification: not applicable	Solicitation: not available
Initial Funding Date: 1996	Expiration: 1997
Students Funded Under Research: 3	Post-Doctoral Associates: 3
FY 1997 Funding: not available	
Joint Agency Participation: NIH and Human Brain Project	

Task Description:

To facilitate the implementation of the software development, an Administration Core will develop and coordinate the computer technologies among the different research projects. The Core will work closely with all five projects in developing not only the precise kinds of equipment that each project needs to complete its scientific goals, but to produce a common foundation of technology to be used by the projects in order to produce a seamless integration of communication and data among the projects. As new enabling and emerging technologies in computers, networking, and communications occur in the computer sciences and in the software developments of this P20, the Core will have the responsibility to evaluate their potential application to the science being conducted by the projects. The Administrative Core will have four specific aims: (1) the Core will be responsible for connecting all computer platforms in each of the four projects of this Consortium together. Both TCP/IP and DDP protocols will be fully supported such that all clients, servers, and applications that the Consortium will develop can be executed or accessed from any computer in the Consortium and to other investigators outside the consortium as software development refinements are found acceptable; (2) the Core will work with other projects in developing computer technologies. Specifically, these are the NeuroZoom systems of the Morrison Project, and the NeuroBase, NeuroAtlas, and NeuroNet systems of the Bloom/Young Project. These technologies will then be distributed to all other projects of this Consortium and to other investigators outside the Consortium as software development refinements are found acceptable; (3) the Core will work with the other projects in incorporating their data into electronic form suitable for NeuroZoom, NeuroBase, NeuroAtlas, and NeuroNet. Specifically, this includes the Jones Project for the electronic digitization of the Jones and Berman *Macaca mulatta* brains and the Bloom/Young Project for the production of the electronic Mannen cat brain atlas; and (4) the Core will work closely with other collaborating P20 and RO-1 applicant groups responding to the Human Brain Project Program Announcement to create the necessary data filters, communication interfaces, and data structures with the overall goal of inter-laboratory data sharing and communications. Thus, the Administrative Core will be specifically responsible for implementing (distributing, installing, and training) the data acquisition, data analysis, database, and communication software as they are developed and for assuring that the collaborating projects can use them appropriately.

Studies and Results: Year 4 has concentrated effort on two issues: (1) completing the NeuroZoom software application for distribution into the scientific community, and 2) using NeuroZoom to create rudimentary brain atlases that could be used for other experiments and incorporation into a database.

Completing the NeuroZoom software: NeuroZoom is a microscopy data acquisition and analysis software program for the Apple Macintosh computer system, designed to support tissue mapping from any kind of microscope using traditional topographical mapping techniques and with statistical stereological probes. NeuroZoom was designed from the ground up to be first and foremost a mapping program. By controlling the movement of the XYZ axes on the microscope and displaying live video images in a computer window with data aligned on it, NeuroZoom is capable of extracting out 3D data from any visualized object. Typical uses of this are creating distribution maps of molecular markers on sections of biological structures. A topographical map captured with NeuroZoom (the delineation of the boundaries) can be coupled to stereological analyses (the rectangular mapping frames are systematically and randomly dispersed over the cortical layers) at different levels of resolution; the raw video images can also be stored in this database and connected in proper spatial context to the reduced neuroanatomic data.

In Year 4, stereological probes have been added to NeuroZoom to assist in the unbiased quantification of cellular counts, nuclear volumes, cellular volumes, and surface areas. The database engine developed in Year 3 has been implemented into the architecture of NeuroZoom so that collected data may be published outside of the local computer that NeuroZoom is running on to other collaborators via the Internet. In addition, a rudimentary framework for a neurocircuitry database is being prototyped with this database engine that focuses on cellular morphology of brains, and the interconnections among them. Using either NeuroZoom itself, or another software application developed specifically as a client application to this database, Internet users can log into collaborative databases to view collected, synthetic, and reduced data. Models include raw data based on topographical maps, stereological estimates of counts, surface areas, and volumes, and of connectational information that arise from both conclusions drawn from experiments conducted with NeuroZoom, as well as from encyclopedic investigations of knowledge in the database or from the literature. All software development was done by Dr. Warren Young (Scripps), also directing all programming efforts, and the programmers, Soraya Gonzalez (Scripps) and Harry Stern (Mount Sinai).

Significance: NeuroZoom has been installed at the Department of Neuropharmacology, Scripps Research Institute, Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, Department of Neurosciences, University of California, San Diego, Washington University, St. Louis, and Rush Presbyterian Hospital in Chicago. We had a successful showing at the 1996 Society for Neurosciences meeting in Washington, DC, and have created a specialized Web site to support the distribution and use of NeuroZoom. Given the interest expressed at our abstract poster displays at the 1996 Society for Neuroscience meetings, we expect that there will be many scientists downloading and using the software. Since NeuroZoom was designed for overall general use in topography and stereology, scientists from outside of basic neurosciences will also be encouraged to use the software. We have compared NeuroZoom to 4 other commercial packages during the 1996 annual meeting of the Society for Neurosciences, and have found that the feature set in NeuroZoom is far more complete and advanced than all others. The stereology is coupled to topographical mapping, which only one other commercial software, BioQuant, can claim as a feature. However, BioQuant, at \$18,000, provides only one stereological tool, the optical dissector, for estimating cell counts, while we provide tools for global and regional volumes and surface areas. Furthermore, we are the only software package that is coupled to a networked database that can act as a locally- or globally-accessed system.

The work on NeuroZoom as an acquisition tool using stereological principles, and the development of a database with stereological data has also led to the submission of a STTR grant proposal by Floyd Bloom. A stereologically-derived database of key mouse brain structures in the normal brain will be developed and compared against transgenic mouse models of spinal neurodegeneration. The intent is to provide a normative model for pathology in genetically-manipulated mice using unbiased stereological data.

We feel that the contributions from use of NeuroZoom will be immense, providing easy to use stereology tools to collect data in an unbiased manner. The *Journal of Comparative Neurology* recognizes the importance of collecting data in an unbiased manner, and is requesting that all papers submitted to their journal use these techniques when collecting quantitative data. These data may then be compared in similar unbiased manner to data from other collaborators, and contributes to a growing database of quantitative neuroanatomic knowledge.

Plans: Year 5 will see the release of NeuroZoom into the scientific community. Documentation is being completed, existing software bugs are being eliminated, and a support system is being put into place as part of the specific aims of the Administrative Core. Currently, support for NeuroZoom is very important. This is not a small, simple application. NeuroZoom controls electronic equipment, such as motorized microscope stage systems. The microscope needs to be properly configured and connected to the computer running NeuroZoom. Furthermore, if the stereological protocols are to be used, a good strong working knowledge of stereological principles are required in order to produce the best results. To this end, Dr. Esther Nimchinsky and Dr. Patrick Hof are writing up documentation on the principles of stereology, and how to use stereology within the context of NeuroZoom. Both basic principles and task oriented chapters are being written. These and the basic manuals to support other features of NeuroZoom are in a portable document format that any computer system can open. Documents are retrievable from the NeuroZoom Web site, and are also viewable directly from the Web browser.

MORRISON SUBCONTRACT - PROJECT II

Studies and Results: The NeuroZoom package for quantitative microscopy and neuroanatomy has continued to progress with new modifications incorporated and tested extensively in our laboratory (see Dr. Young's description for details on software). Extensive software development occurred through an impressive collaborative effort across the Mount Sinai and Scripps components in which the programming was directed by Dr. Young, and the testing of programs and microscopic analysis being carried out primarily at Mount Sinai. Dr. Nimchinsky has worked very closely with Dr. Young and his team on the development of new stereological tools. The major capabilities of NeuroZoom are the following: (1) Full computerized, unbiased stereological procedures for neuron counts, volume, size, and surface area, as well as volumes of specified brain regions; (2) Capacity for tracing dendritic or axonal processes and displaying in 3-dimensions; (3) The automatic display of cell reconstruction data as a dendrogram that is quantitatively accurate in regard to dendritic branching patterns and length; (4) The capacity to make very large scale maps of cell distribution; (5) The capacity to reconstruct the pattern of anterogradely-labeled axons in the context of superimposed electrophysiological recording maps; and (6) The capacity to display a reconstructed neuron within the context of a lower magnification map of cell distribution. In addition, confocal data can now be placed into the context of NeuroZoom reconstructions that have been taken off the Zeiss Axiophot.

Significance: These results are significant on several levels. First, they demonstrate that the use of computer-assisted microscopy to generate detailed and rigorous quantitative data allows the neuroanatomist to ask and answer questions that would not be feasible with more conventional methods of data collection. In this respect, much of the progress over the last two years offers compelling examples of the power of quantitative neuroanatomy, and also offers a means to achieve such power. In addition, on the neurobiological side, much of the data that emerged over the last year have had major implications for the cellular and molecular determinants of vulnerability in neurodegenerative disorders such as Alzheimer's disease and ALS. Through genetically manipulated mice and experimentally manipulated monkeys, we have been able to develop a much more detailed profile of the neurons that are vulnerable to degeneration in both Alzheimer's disease and ALS, and these data will now serve as an important baseline for the development of methods of intervention that might protect these neurons.

Plans: Our plans for the coming year involve both further development of the programs that constitute NeuroZoom as well as continued use and refinement of this software to develop more detailed neuroanatomic and cellular data. The quantitative analysis of glutamate receptor distribution will figure prominently in the neurobiological studies to be performed, and we will extend our analyses of transgenic mice, particularly stereological analyses of mouse models of selective vulnerability and neurodegeneration. In respect to

NeuroZoom development, we will continue to refine the software available for light microscopy while concentrating on developing software mechanisms for 3-dimensional display of reconstructed neurons and the integration of quantitative data across ultrastructural confocal and light microscopic analyses. In these respects, we intend for NeuroZoom to not only become a more refined package that will be able to be distributed to Beta test sites, but also, to have much broader capabilities in respect to high resolution microscopy.

KARTEN SUBCONTRACT - PROJECT III

Studies and Results: Retinal Database: We are proceeding with the development of the formal interface using *Illustra*. However, due to recent corporate pricing policy at *Illustra* and the need for a low cost database for the average laboratory, we have continued to expand the use of FileMaker Pro 3.0 as a relational database. We have started to load the database with such material as retinal cell types, transmitter information, as well as confocal and other retinal images.

Classification Schemata for 3D Morphology of Retinal Neurons: We have developed a number of new tools for analysis of 3D datasets of filled retinal neurons. Cells are being reconstructed, and we are testing various "objective" classification schemes of the 3D morphology for use in a database. These include use of *fractal*, *multifractals*, and *moments*. Each has proven of some value, and the final classification is likely to include a multidimensional collection of information using these and other criteria (e.g., lamina disposition of *fractal* types of a multistratified neuron).

Quantitative Methods in Microscopy: The earlier version of our manual on post-acquisition processing of confocal images using NIH-Image has been extensively revised. It now includes a large collection of tools as macros for collecting very weak fluorescent images, and a new powerful set of tools for analysis of confocal images, including generation of 3D raytracing images, double and triple labeled Z-series, rotational 3D series, and other utilities of value to quantitative and qualitative confocal microscopy. The manual, macros, and database files are posted on the NIH-Image site at NIH as well as on our local server.

Plans for the coming year: Retina Database: Our current emphasis is on the development of schemata for 3D classification of detailed dendritic morphology of retinal neurons. We have completed an initial Policy Guideline and are proceeding with both the fractal and moment analysis. We now plan to link this directly to our confocal data collection of 3D datasets of filled retinal ganglion cells.

The Database will be available for online searching from the Website using FileMaker Pro WebServer

Quantitative Confocal Microscopy: Using tools developed during the past year, we now are able to generate 3D images of filled cells. We hope to obtain a dedicated confocal microscope for this work to demonstrate the feasibility of doing this online - i.e., filling cells, collecting confocal images, and classifying the cell type based on linkage to the database while still recording from the ganglion cell. This requires continuous access to a confocal microscope attached to the cell filling/recording rig. We request permission for re-budgeting for the requisite purchase of a confocal unit.

JONES SUB-CONTRACT (ORIGINALLY PROJECT V)

In the past year, we have continued to scan our collection of serially-sectioned monkey brains, cut in the three standard planes, and three human thalami cut coronally, sagittally or horizontally. The monkey brain sections are stained with thionin. The human sections are stained with thionin or for acetyl cholinesterase which gives extremely good resolution of nuclear borders.

All images which continue to be obtained with the Nikon LS-35 10AF film scanner, are saved as TIFF files. Each image, even with some degree of compression commensurate with preservation of detail, amounts to 65-125 megabytes so the process is a slow one and storage, especially storage in a form compatible with export to other scientists is a problem. In the last year, we have written all our files to CDs, using a conventional CD writer. Each CD, nevertheless, can rarely accommodate more than three images.

A complete series of scans of the acetyl cholinesterase stained sections from three human thalami have now been completed and the files stored on CDs. Scanning the Nissl series remains in progress and continues at a pace that we wish were faster but is limited by the equipment. We have made digital equivalents of camera lucida drawings of the human thalamic scans and are currently making them available to interested parties, primarily for merging with MRI and PET scanned images of the human thalamus. Currently interested other groups are those of Dr. Arthur Toga at UCLA, Dr. Monte Buschbaum at Mt. Sinai, and Dr. Nick Bryant at Johns Hopkins.

In the coming year, we will have completed all the monkey brain scans and should be well on the way toward completion of the human scans. The autoradiography of receptor mRNAs should also be finished. We are faced with the task of how best to make the digital thalami available to other scientists. The unadorned scans are available now via CD but we feel that it is essential to provide some sort of outline atlas-type drawing to accompany them.

This research seeks to understand the common elements that can lead to loss of brain functions by death of neurons. This work can define how individual subjects may become vulnerable or resistant to environmental challenges that can accelerate death of neurons. Furthermore, the technology developed through this research can offer new ways to link chemistry, anatomy, physiology, and treatments with specific cellular components of the brain.

FY97 Publications, Presentations, and Other Accomplishments:

Hof, P.R., Ungerleider, L.G., Adams, M.M., Webster, M.J., Gattass, R., Blumberg, D.M., and Morrison, J.H. "Callosally projecting neurons in the macaque monkey V1/V2 border are enriched in nonphosphorylated neurofilament protein." *Visual Neurosci.*, 14, 981-987 (1997).

Hof, P.R., Ungerleider, L.G., Webster, M.J., Gattass, R., Adams, M.M., Sailstad, C.A., and Morrison, J.H. "Neurofilament protein is differentially distributed in subpopulations of corticocortical projection neurons in the macaque monkey visual pathways." *J. Comp. Neurol.*, 376, 112-127 (1996).

Jacobson, S., Henriksen, S.J., Prospero-Garcia, O., Phillips, T.R., Elder, J.H., Young, W.G., Bloom, F.E., and Fox, H.S. "Cortical neuronal cytoskeletal changes associated with FIV infection." *J. Neurovirol.*, 3, 283-289 (1997).

Jones, E.G. "The thalamus of primates" in "Handbook of Chemical Neuroanatomy, Volume 13B Primate Brain." Edited by: Bloom, F.E. Elsevier, Amsterdam (In Press).

Morrison, B.M., Gordon, J.W., Ripps, M.E., and Morrison J.H. "Quantitative immunocytochemical analysis of the spinal cord in G86R superoxide dismutase transgenic mice: Neurochemical correlates of selective vulnerability." *J. Comp. Neurol.*, 373, 619-321 (1996).

Nimchinsky, E.A., Hof, P.R., Young, W.G., and Morrison, J.H. "Neurochemical, morphologic and laminar characterization of cortical projection neurons in the cingulate motor areas of the macaque monkey." *J. Comp. Neurol.*, 374, 136-160 (1996).

The Role of Vestibular Information in Adaptive Modification of Eye, Head, and Hand Coordination

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Funding:

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Students Funded Under Research: 0
FY 1997 Funding: \$ 111,000

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Expiration: 1998
Post-Doctoral Associates: 2

Responsible NASA Center: Johnson Space Center

Task Description:

The central nervous system (CNS) integrates multisensory information to determine body spatial orientation relative to the environment. Exposure to the microgravity conditions encountered during space flight induces alterations in this internal construct producing perceptual and sensory-motor disturbances during adaptation to microgravity and readaptation to a 1-G environment. Accurate ocular and manual localization of targets in extrapersonal space requires the proper integration of sensory input. The ability to accurately coordinate eye, head, and hand movements is essential for safe shuttle operations; however, little is known about the role adaptive alteration in vestibular input plays in the coordination of eye, head, and hand movements. Therefore, the first objective of this ground-based study is to determine the role vestibular spatial coding plays in the formulation of goal-directed eye and hand localization of targets. The second objective is to determine if adaptive alterations in eye-head coordination produce commensurate alterations in the ability to manually locate target positions, and conversely, if adaptive modification in eye-hand coordination transfers to the eye-head system. This investigation will help elucidate the basic mechanisms underlying the spatial programming of coordinated eye, head and hand movements along with their adaptive properties. This basic information will be used for the design of similar investigations for space flight.

The results of the first two experiments provide insight into whether vestibular information can be used to spatially code goal-directed manual localization of remembered targets in darkness. We compared subjects ability to point accurately at a fixed target displayed on a plain background versus a featured background. We then compared subjects pointing accuracy following whole-body rotation. We presented the results of the second study at the 1995 Society for Neuroscience Annual Meeting in San Diego, California. We also presented some preliminary data at the Life Sciences and Space Medicine conference, sponsored by the American Institute of Aeronautics and Astronautics (AIAA) held in Houston, Texas in April 1995. A paper entitled "A system for the accurate measurement of pointing responses" was published in the *Journal of Neuroscience Methods*, describing the system used in our lab for accurately measuring pointing responses.

The results of our third study provide empirical evidence that adaptive alteration to VOR function produces corresponding alterations in vestibular contingent pointing responses. Subjects were exposed to a 30 minute adaptive stimulus to modify VOR function. A comparison of pre and post-adaptation responses showed a significant decrement to pointing performance in successfully locating target position after the adaptive stimulus. To confirm that these results were based on vestibular cues, we tested two bilaterally labyrinthine deficient (LD) subjects using this testing paradigm. LD subjects were unable to accurately locate an Earth-fixed target after passive whole-body rotation, confirming the hypothesis that vestibular information is essential in successful performance of a vestibular contingent pointing test. These results were presented in poster format at the 1997 Society for Neuroscience Annual Meeting in New Orleans, Louisiana.

Our fourth experiment is underway to determine if adaptive alterations in eye-hand coordination transfers to the eye-head system. These results suggest a "top-down" hierarchy of adaptation such that adaptation occurs from proximal to distal body segments.

Through these investigations, we have answered the following questions: Will differences in the visual context of target display affect subjects' pointing accuracy? Can vestibular information alone be used to spatially code manual pointing responses? Can adaptive alterations in eye-head coordination produce commensurate alterations in the ability to manually locate target positions? What is the role of the vestibular signal in the spatial coding of saccadic eye and manual pointing movements? Can adaptive alterations in eye-hand coordination produce commensurate alterations in eye-head coordination?

The results of these experiments will serve as the foundation for our on-going investigation aimed at determining how the oculo-motor and manual systems share information, and how this information is susceptible to common adaptive distortion following exposure to conflicting visual-vestibular stimuli.

Development of experimental paradigms that attempt to delineate canal and otolith contributions to motor control has both fundamental scientific importance and potential practical applications. Our experiments are yielding results that may be compared and contrasted with the impairment experienced by the elderly or clinical populations. The investigation of neural adaptation to microgravity will lead to better understanding of neural alterations associated with aging and other neurological disorders. The development of unique research protocols to investigate neural alterations in the control of gaze can aid clinicians in diagnosis of neurological and neurovestibular pathology and in monitoring post-surgical recovery.

One main goal of the research conducted in our laboratory is to characterize how the central nervous system integrates multi-sensory information to determine the spatial orientation of the body in space. This research examines how various neural systems adaptively respond to changes in the relationship between sensory input and motor output. Ultimately, we will understand how these systems adaptively respond to the sensory conflict conditions of space flight. The development of a basic understanding of the underlying mechanisms involved in the adaptation process will aid in the identification and testing of countermeasures that will reduce or eliminate the risk associated with these neural adaptive changes.

What relationship does this task posit between processes on Earth and in space? Exposure to the microgravity environment of space flight induces adaptive modification in the central processing of sensory input to produce motor responses appropriate for the prevailing gravito-inertial environment. Development of experimental paradigms that attempt to delineate canal and otolith contributions to motor responses has both fundamental scientific importance and potential practical applications. Adaptive reinterpretation of otolithic input has been hypothesized as a major contributing factor to postflight motor control problems. Understanding how the canals and otoliths integrate information concerning body motion in a 1-G terrestrial environment will enable predictions and hypotheses to be made concerning how this interaction is modified following exposure to microgravity conditions.

The development of a unique research protocol to determine how normal subjects adapt to altered sensory information can be used by clinicians to develop enhanced rehabilitation techniques for patients with balance

disorders saving billions of dollars in health care expenditures. Development of this new technology can lead to the establishment of worldwide clinical vestibular testing norms that can be used in medical facilities. In addition, this research can lead to the formulation of models of neural activity based on known pathways and substrates. These models can be used to make predictions about response properties and transfer effects of a variety of motor subsystems following exposure to microgravity or as a predictive tool for clinical populations.

Biochemical Adaptations of Anti-Gravity Muscle Fibers to Disuse Atrophy

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Funding:

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Post-Doctoral Associates: 5

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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

An original aim of this research was to determine the DNA sequences that are involved in altering promoter activity of genes in non-weight bearing (unloaded) skeletal muscles. We initially proposed to investigate the skeletal α -actin promoter, and still intend to do so. However, we have tested two other genes not initially proposed. First, based upon findings in our laboratory from a NIH grant, we tested a 3'-UTR region of the cytochrome c mRNA whose RNA-protein interaction had been shown to be: a) decreased when contractile activity of low oxidative muscle was increased, and b) low in the soleus muscle compared to a low oxidative muscle. Second, we received transgenic mice expressing the promoter of the human slow troponin I gene from Dr. Hardeman. We established hindlimb non-weight bearing of mice to perform transgenic mice experiments. Our second initial aim was to determine whether an increased expression of insulin-like growth factor (IGF-I) within the muscle can serve as a countermeasure to attenuate or prevent atrophy during non-weight bearing. We have tested this aim in transgenic mice. Our initial results are promising, but require more experiments.

We have examined the regulation of the troponin I s (TnIs) promoter during skeletal muscle unloading-induced protein isoform transition, using a transgenic mouse line harboring the -4200 to +12 bp region of the human troponin TnIs promoter. 18 female transgenic mice (about 30 grams body weight) were randomly divided into two groups: 1) weight bearing (WB) controls, N = 9, and 2) hindlimb unloaded (HU), N = 9. The HU group were tail suspended for 7 days. Body mass was unchanged in the WB group, but was reduced (about 6%; $P < 0.05$) following the HU treatment. Absolute soleus muscle mass (about 25%) and soleus mass relative to body mass (about 16%) were both lower ($P < 0.05$) in the HU group compared to WB mice. Northern blot analyses indicate that 7 days of HU results in a 64% decrease ($P < 0.05$) in abundance of endogenous TnIs mRNA (micrograms/mg muscle) in the mouse soleus. Further, there is a trend for the abundance of the fast TnIs mRNA to be increased (about 34%). Analysis of transgenic CAT activity in the soleus muscle revealed no difference between WB and HU groups. We conclude that additional elements are necessary for the TnIs gene to respond to an unloading-induced slow-to-fast isoform transition stimulus.

The size of skeletal muscle determines the ability to perform manual work. Skeletal muscle loses one-half of its mass by the age of 80 years in humans. In many cases, this results in humans losing their ability to care for themselves, i.e., they do not have the ability to accomplish the activities of daily living. Humans lose 10% of

their muscle mass from ages 25 to 50 years and lose an additional 40% of their muscle mass from ages 50 to 80 years. In the model of hindlimb non-weight bearing, the amount of muscle mass lost in years in humans is condensed to weeks. After one week of hindlimb non-weight bearing, mice have losses of 10–20% in skeletal muscle mass. Space flight also offers a laboratory to accelerate the loss of muscle mass and to determine why humans lose muscle mass with aging. Loss of skeletal muscle also occurs in many illnesses, such as AIDS, diabetes, obesity, congestive heart failure, etc. NASA studies into muscle atrophy can be considered as nearly the sole source for this research problem as NIH supports little research into muscle atrophy.

This research is also attempting to determine whether the upregulation of IGF-I expression could be a countermeasure to muscle atrophy produced by non-weight bearing of muscle. If successful, IGF-I would be a new therapeutic for preventing muscle atrophy on Earth. If methods of prevention of skeletal muscle atrophy can be found for humans, the quality of life would be enhanced by delaying the entry of people into nursing homes because of physical frailty and by speeding the rehabilitation of skeletal muscle during many clinical diseases. An additional benefit is the reduction of health care costs, a problem which will increase as more Americans reach the age of frailty.

FY97 Publications, Presentations, and Other Accomplishments:

Booth, F.W. and Criswell, D.S. "Molecular events underlying skeletal muscle atrophy and the development of effective countermeasures." *Int. J. Sports Med.*, 18, S1-S5 (1997).

Criswell, D.A., Hardeman, E.C., and Booth, F.W. "The nerve-responsive troponin I slow promoter does not respond to unloading." *J. Appl. Physiol.* (In Press).

*New Statistical Methods for Immunoassay Data Analyses***Principal Investigator:**

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Task Description:

The broad, long-term objectives of this project are to: (1) develop more accurate statistical methods for immunoassay data analysis by devising a unified framework for quality control, calibration, and measurement based on Bayesian statistical theory; (2) contribute to biomedical knowledge by establishing a new paradigm for monitoring and maintaining quality control of biotechnologic processes; and (3) enhance biomedical knowledge of physiologic adaptation to space by ensuring the validity and interpretability of endocrinologic data collected on manned space missions and in Earth-based space environment simulations.

The specific aims are to: (1) establish experimentally sound and statistically rigorous methods of integrating into a single paradigm immunoassay quality control, calibration, and measurement (QCCM) by applying Bayesian statistical theory; (2) devise computationally efficient algorithms for QCCM which will allow the new paradigm to become a standard part of routine laboratory performance monitoring, immunoassay data analysis, and result reporting; (3) compare the performance of the QCCM paradigm with established quality control and data analysis paradigms using several different types of immunoassay systems; and (4) implement on a Windows 95 platform a numerically efficient immunoassay data analysis software package based on the QCCM model and the Bayesian paradigm which can be transported on a space mission or to any ground testing site.

The experimental design and methods used are: (1) theoretical work to design the statistical models; (2) empirical studies of immunoassay experimental data; and (3) computer simulations to investigate the properties of the immunoassay experiments, the statistical models, and the mathematical algorithms.

The health-related implications of this study are far-reaching in that the methods developed here offer a means of performing more accurate immunoassay data analyses in any clinical or research laboratory, and for monitoring and maintaining quality control of biotechnologic processes.

What has been accomplished thus far (and particularly during FY97)?

During FY97 we completed implementation of a maximum likelihood algorithm for estimating the model parameters and computing empirical Bayes' estimates of the analyte probability densities. We completed testing of the algorithm on a wide arrange of data sets from several different types of assays. The computer program is self contained and is written in the C programming language.

The second part of the project on which we made substantial progress during this year is on the further development of our quality control calibrations and measurement algorithm. This algorithm is designed to establish a unified framework for executing these 3 tasks. The algorithm, as previously described, works very well for combining information across several assay experiments. The algorithm provides a rigorous way for establishing initial quality control conditions based a set of assays experiments during which the assay process is considered to be in control. It next provides a new statistical approach for testing the agreement of a new assay with the quality control criteria. This testing is carried out in formal statistical framework using Bayesian predictive densities. Once it is determined that the new assay experiment is in control, the algorithm uses Monte Carlo Markov chain methods to update the posterior distribution of the parameters and for calculating the estimates of the analytes based on the new assay experiment.

The Bayesian statistical procedures which we have developed for sequential computation have been applied not only to the analysis of immunoassay data but to the problem of decoding physical variables represented in neural firing patterns as well. In this problem, similar computational issues arise which can be readily resolved by appropriately modifying the sequential algorithms we have developed through our immunoassay research. We have completed two technical reports on this work.

Our other accomplishments to date include: (1) the publication of our manuscript on the analysis of plasma melatonin levels in which we used our Bayesian statistical methods to accurately incorporate the immunoassay error into that model; (2) publication of a chapter in the upcoming edition of Wilson Endocrinology detailing current procedures for immunoassay data analysis and presenting our new methods; and (3) a patent, issued this year, on our procedure for immunoassay data analysis and assay calibration.

What new questions have arisen?

The area where new problems have arisen is in determining the accuracy of the estimates of the analyte concentration. At present, the estimates which we obtain from our full Bayesian procedure are significantly more variable than those obtained from the empirical Bayes or classical estimates. We are therefore, currently studying a Kalman filter modification to algorithm to obtain more correct estimates of the variability in the analyte determinations. We believe that this problem will be resolved soon.

How does this fiscal year's progress affect future work on this task?

The progress this years suggests a well defined approach to making more efficient use of data from immunoassay experiments. We will continue to develop this approach in the coming years.

The research represents the development of new statistical techniques to analyze immunoassay data. These methods should have broad applications in clinical and laboratory medicine because immunoassays are the most widely used procedure for measuring the concentrations of analyte in biological specimens. The primary benefits of these new methods will be on Earth; however, they may be used to analyze immunoassay-based measurements of biological specimens collected during space missions.

The new technologic benefits from the research include: (1) a new method for determining the accuracy of any immunoassay measurement; (2) a new method for setting standards for immunoassay quality control; (3) a new approach for accurately defining the smallest concentration an immunoassay can measure; (4) new criteria for optimal design of immunoassays; and (5) PC-based software to apply the new methods. The health-related benefits of the new methods apply to any immunoassay based procedure. They are the establishment of more statistically rigorous standards for defining positive test results for disease screening and diagnostic medical tests, and for measuring reliably any analyte concentration with an immunoassay. Because the measurement of

analytes with calibrated methods (spectroscopy, chromatography, and quantitative PCR) is an important problem in many scientific disciplines, our statistical paradigm should be applicable to other analytic procedures as well.

FY97 Publications, Presentations, and Other Accomplishments:

Patent Approved, U.S. Patent #: 5,616,504 Brown, E.N. and Skates, S. "Methods and system for calibration of immunoassay systems through application of Bayesian analysis."

Brown, E.N. and Yuan, A. "A probabilistic expert system for immunoassay quality control, calibration and measurement." Technical Report 94-01, Statistics Research Laboratory, Department of Anesthesia and Critical Care, Massachusetts General Hospital, Boston, MA, 32 pages (revised September, 1997).

Brown, E.N., Choe, Y., Czeisler, C.A., and Shanahan, T.L. "A mathematical model of diurnal variation in plasma melatonin levels." *Am. J. Physiol. (Endocrinol. Metab., 35)*, 272, E506-E516 (1997).

El-Hajj, F.G., Klerman, B., Brown, E.N., and Czeisler, C.A. "N-Tx durnal rhythm is truly endogenous." (Poster) Annual Meeting of the American Society of Bone and Mineral Research, Minneapolis, MN (1997).

El-Hajj, G., Klerman, E., Brown, E.N., Choe, Y., Brown, E.M., and Czeisler, C.A. "Parathyroid hormone circadian rhythm is truly endogenous." *J. Clin. Endocrinol. Metab.*, 82, 281-286 (1997).

Sergé, G.V., Juppner, H., and Brown, E.N. "Measurement of hormones" in "Williams' Textbook of Endocrinology." Edited by: Wilson, J.D., Foster, D.W., Larsen, R., and Kronenberg, H. 9th Edition (1997).

The Biomechanics of Exercise Countermeasures

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Funding:

UPN/Project Identification: 199-26-17-11

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Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$137,378

Task Description:

Space flight can lead to a significant bone loss and to muscle atrophy. To date, no effective countermeasure has been identified for either of these undesirable effects. There are strong indications, however, that exercise will form a crucial part of any protocol to minimize the adverse effects of space travel. It is hypothesized that an effective exercise regimen should elicit loads on the lower extremities and require muscle actions that resemble those encountered in 1-G.

The objectives of the proposed study are to use a ground-based simulator of zero gravity to define exercise countermeasures in terms of their similarity to 1-G loads and patterns of muscle activity. This will eventually lead (in a subsequent proposal) to a logically planned in-flight experiment in which the efficacy of the proposed exercise program is studied directly in terms of its effect on muscle and bone mass.

The Penn State Zero-gravity Locomotion Simulator (PSZS) is a device designed to enable the collection of ground reaction force profiles from human volunteers who are suspended in a horizontal plane by a system enabling unrestricted locomotion exercise on a treadmill mounted on a wall perpendicular to the force of gravity. This project uses the PSZS to answer three basic hypotheses about the efficacy of specific exercise countermeasures for bone loss during spaceflight and long-term exposure to 0-G. The hypotheses being tested in this research are: (1) There will be no differences in peak ground reaction forces and peak loading rates between overground gait and gait in the full bodyweight loaded conditions in the PSZS; (2) There will be no differences in hip, knee, and ankle joint positions between walking or running overground, on a standard treadmill, and in full bodyweight loaded conditions in the PSZS; and (3) The muscular activations, as a percentage of maximal voluntary contraction, will be similar between walking or running overground, on a standard treadmill, and in full body-weight loaded conditions in the PSZS.

During this funding period, data has been analyzed from 16 subjects during walking and running in four experimental conditions: (1) normal overground locomotion in 1-G; (2) locomotion on a conventional treadmill in 1-G; (3) locomotion with tethering springs attached only at the subjects' shoulders to provide simulated full bodyweight gravitational force replacement (FBGFR) in simulated 0-G using the PSZS; and (4) locomotion with the tethering springs attached at the subjects' waists and shoulders to provide FBGFR in simulated 0-G using the PSZS. Comparisons of the profiles of ground reaction forces, muscular activations, and joint kinematics from each of the four conditions now show that significant differences can be identified in each of

these three profiles when comparing 0-G and 1-G conditions. Further consideration of these discovered differences in conjunction with assessments of comfort of the 0-G tethering system suggest that the differences are most likely the result of dissimilar bodyweight load distribution patterns between the 0-G and 1-G conditions as discussed below.

Ground Reaction Forces: Maximum active ground reaction force peaks were significantly larger in 1-G than in the 0-G condition counterparts. Two mechanisms may explain the differences: (1) increased knee flexion as was noted during the 0-g conditions (as described below), and (2) fluctuations in subject loading during running with gravity replacement in 0-G. It was also noted, however, that ground reaction force profiles did appear to be remarkably similar when normalized to subject load instead of body weight.

Muscular Activations: The tibialis anterior, rectus femoris, and vastus lateralis had significantly greater activation integrals in both 0-G conditions when compared with 1-G conditions. No differences were found in the activations of the gastrocnemius, biceps femoris, and gluteus maximus.

Joint Kinematics: No differences were found in the motion of the hips and ankles between the 0-G and 1-G conditions; however, the knees were significantly more flexed in 0-G than 1-G during both loading conditions.

The influence of the gravity replacement load on the perceived comfort, knee kinematics, and ground reaction force variables was the most important finding of this study. This influence was present in the mechanical aspects of load fluctuation and the psychological aspects of discomfort or heightened consciousness of the load. While load fluctuation had a dramatic effect on the ground reaction forces, the desirability of this large fluctuation will remain to be determined after it is known whether large forces or large loading rates are more important in the maintenance of bone density. However, harness comfort will need to be carefully considered in future exercise countermeasure recommendations. Maximization of harness comfort is of utmost importance if subjects are to run for extended bouts of exercise over a period of several months.

During this period, work has also been conducted on the study of a vibration-isolated treadmill for use in the International Space Station. Quantitative video data were collected during STS-81 and analyzed to determine the stability of the system.

Although the primary impetus for this research is to design exercise countermeasures to address the problem of bone loss during long-term space flight, knowledge gained from this research will provide crucial insight into the importance of exercise for the development and maintenance of bone strength among humans living on Earth in "normal" gravitational fields. Moreover, a fully-validated PSZS will provide a means of studying the role of physical loading in the development and regulation of the human skeletal system. This system will be useful in future studies of both short-term and long-term bone strength problems including pathologies affecting osteogenesis in adolescents and the issue of osteoporosis in older adults.

In the future, the PSZS will enable research that goes beyond the design of exercise countermeasures. The PSZS will provide a means of studying the secondary signaling systems that convert physical stimuli such as ground reaction forces into the biochemical signals that directly control the human skeletal system. Knowledge in this area is crucial to the treatment of bone disease for which exercise may not be an effective or reasonable intervention.

FY97 Publications, Presentations, and Other Accomplishments:

Cavanagh, P.R. "The foot-ground interface: Modeling and experimentation." (Abstract of ISB President's message) Presented to the 16th Annual Meeting of the International Society of Biomechanics (ISB), Tokyo (August 25 - 29, 1997).

McCrary, J.L., Baron, H.A., Derr, J.A., Davis, B.L., and Cavanagh, P.R. "Subject load-harness interaction during zero-gravity treadmill exercise." (Abstract) Presented to the 20th Annual Meeting of the American Society of Biomechanics, Georgia Institute of Technology, Atlanta, GA (October 17 - 19, 1996).

Thornton, W.E., Cavanagh, P.R., Buczek, F.L., Milliron, M.J., and Davis, B.L. "The kinematics of treadmill locomotion in space" in "Three-Dimensional Analysis of Human Locomotion." Edited by: Allard P., Cappozzo A., Lundberg A., and Vaughan C.L. John Wiley & Sons (In Press).

Gravity in Human Oculomotor Control, Perception and Action

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Funding:

UPN/Project Identification: 199-16-12-40
Initial Funding Date: 1994
Students Funded Under Research: 7
FY 1997 Funding: \$207,000

Solicitation: 93-OLMSA-07
Expiration: 1997
Post-Doctoral Associates: 2

Responsible NASA Center: Ames Research Center

Task Description:

Perceptual illusions and degraded psychomotor performance result during and after exposure to the unusual gravitational-inertial conditions encountered in space flight. Because these illusions and disruptions of behavior can compromise safety and because they are important both theoretically and practically, we are attempting to enhance our understanding of them.

The perceived location of visual targets depends on both retinal and extra-retinal information. Both retinal stimulation and stimulation of the vestibular organs affect oculomotor control, which in turn, influences perception and spatially-directed behavior. Although quantitative relationships among these variables can be determined under specific conditions, the relationships are adaptive, in that the organism can learn to extract meaning under conditions in which it is given an opportunity to interact with the environment. These adaptive processes are such that the organism can learn to function appropriately in an environment in which it did not originally develop or evolve.

The studies all involve the systematic alteration of the visual and/or the gravitational-inertial field in which human subjects perform. Centrifugation, water immersion, and altered visual stimuli are used to determine how human oculomotor control, perception, and perceptual-motor behavior depend on these aspects of the environment, to delineate the range over which normal functioning remains unaffected by these parameters, and to develop quantitative models that describe and predict how oculomotor control, perception, and perceptual-motor behavior are altered by systematic changes of the environment.

We expect that this research will yield the following results: 1) we will increase our understanding of how gravity combines with visual stimulation to influence oculomotor control, perception, and visual-motor behavior; 2) we will document intersensory interactions and feedback mechanisms in perceptual and behavioral adaptation to altered gravity and altered visual stimulation; and 3) we will develop analytic, descriptive, and predictive techniques that enhance our understanding of the underlying mechanisms.

Several of our ongoing research protocols were completed and analyzed during FY 1997, and new protocols were initiated. Although most of the experiments contained in our initial research proposal for this task have been completed, we were unable to conduct some of the others. The relationships among oculomotor control, human visual perception, and action remain largely unspecified, and continue to be of significant scientific and practical importance.

Analyses of data from HR-160, which examined how G-perception is affected by prolonged (up to 1-hour) exposures to 2.0 Gz hypergravity were largely inconclusive. Appreciable ranges in G-tolerance during longer exposure intervals, and the failure of some subjects to tolerate the prolonged exposures, have forced us to reassess both the experimental design and our methodology. We have not yet been fully able to identify or resolve the underlying problems.

We completed the analysis of data from Research Protocol HRII-080, and are scheduled to present these results to the 1997 meetings of the Psychonomic Society. Our data revealed that, if images are initially learned in the erect orientation, reaction times increase as the images deviate from erect. In contrast, if the images are initially learned in the inverted orientation, there is no significant effect of stimulus orientation in subsequent trials. Additional studies investigating the detection of facial affect when the faces are initially viewed in an erect or an inverted orientation were also conducted during the past year. These data, which are still being analyzed, examined the influence of repeated trials on the effects of orientation. The data clearly show that the effects of orientation on reaction time are significantly reduced with repeated presentations of non-vertical stimuli. This finding suggests that appropriate training can be used to diminish the effects of altered stimulus orientation in the real world.

We completed our final study of human perception of the zenith as it relates to oculomotor control (HRII-081). We used our ISCAN camera to record eye position when the orientations of subjects were changed and when they placed a visual target to the apparent zenith. Changes in body orientation significantly affected both target placement and eye position. The results were reported at the 1997 European Conference on Visual Perception in Helsinki.

Research Protocol HRII-082 examined the effects of stimulus orientation during initial learning on subsequent recognition of airport runways when the runways are seen in various orientations. The study demonstrated that discriminative responses to maps of airports were most rapid when the maps were seen in the same orientation as that in which they were initially learned, that reaction time was reduced with repeated stimulus exposures, and that information learned from navigation maps was not sufficient for all observers to recognize aerial photographs of the same airports. A report on these results is currently in press in the journal *Aviation, Space & Environmental Medicine*.

A follow-up study investigating transfer of learning under the same basic protocol was also conducted. The results of this study indicated that transfer from maps to maps or from photographs to photographs were both more effective than transfer from maps to photographs or from photographs to maps. An abstract of this research has been submitted for presentation to the 1998 meetings of the Aerospace Medical Association.

We recently conducted a series of studies investigating the influences of rolled, pitched, and yawed visual frames on judgments of vertical, eye-level, and straight-ahead, respectively (HRII-107). Fifty-two observers made these judgments with all three frames. The results showed that the different frames all had significant influences in biasing judgments, but that the amounts of bias in judging vertical, eye-level, and straight-ahead were uncorrelated with one another. The results bring into question the notion of "field dependence" and "field independence" as valid descriptors of individual perceptual style. We found that observers who were "field dependent" with one visual frame were "field-independent" with other visual frames. The results of this study were submitted as an abstract for presentation to the 1998 meetings of the Aerospace Medical Association.

Research Protocol HR-173 is currently in progress at the Ames 20-G centrifuge facility. The protocol examines the effects of open-loop versus closed-loop pointing on adaptation of hand-eye coordination in 1.0, 1.5, and 2.0

Gz environments. When completed, this study should answer critical questions regarding the role of continuous versus terminal visual feedback on the process of adaptation to altered gravity.

The current research is expected to increase our understanding of how gravity combines with visual stimulation to influence oculomotor control, perception, and visual-motor behavior, both on Earth and in space. This information is important in understanding human spatial orientation and disorientation, as well as how intersensory interactions and feedback mechanisms operate to modify perceptual and behavioral functioning. The development of analytic, descriptive, and predictive techniques will enhance our understanding of the underlying mechanisms that operate in both terrestrial and space environments and under both normal and abnormal physiological conditions. To the degree that our models can be used to describe normal physiological and behavioral capabilities, they can also be used to determine and to quantify deficits in behavior that result from disease states. Finally, these studies are potentially useful in showing how spatially-coded information can best be presented to individuals so that their learning is optimized.

FY97 Publications, Presentations, and Other Accomplishments:

Cohen, M.M. and Mealey, J.B. "Effects of orientation on recognition of facial affect." (Abstract) *Aviat. Space Environ. Med.*, 68, 640 (1997).

Li, J., Cohen, M.M., DeRoshia, C.W., and Guzy, L.E. "Effects of observer orientation on perception of ego- and exocentric spatial locations." *European Conference on Visual Perception, Helsinki (August, 1997).*

Mealey, J.B., Cohen, M.M., and Jorden, K. "Effects of map orientation during learning on airport identification." *Aviat. Space Environ. Med.* (In Press).

Welch, R.B., Cohen, M.M., and DeRoshia, C.W. "Reduction of the elevator illusion from continued hypergravity exposure and visual error-corrective feedback." *Perception & Psychophysics*, 58, 22-30 (1996).

Welch, R.B., Post, R.B., Lum, W., and Cohen, M.M. "Are vision and action dissociated with respect to pictorial illusions?" *National Convention of the Psychonomic Society, Chicago (November, 1996).*

NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis

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Funding:

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Solicitation: 96-OLMSA-01

Initial Funding Date: 1994

Expiration: 2000

Students Funded Under Research: 8

Post-Doctoral Associates: 5

FY 1997 Funding: \$175,000

Task Description:

The NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis is dedicated to the application of quantitative methods to understand the basic mechanisms involved in alterations in cardiovascular functions during and after space flight. As part of this effort, the Center develops new technologies to measure alterations in cardiovascular function and to guide the application of possible countermeasures. Many of these technologies are useful not only in the context of space flight research and space medicine but also have important spin-off applications for clinical medicine on Earth. In fact, a number of technologies developed in the Center are in the process of being commercialized for clinical application. One of these spin-off technologies—a non-invasive diagnostic test for identifying individuals at risk for ventricular arrhythmias and sudden cardiac death—has received FDA approval and is being marketed in the United States, Japan, and Europe. In addition to its research function, the NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis has training and educational components. The Center is involved in training undergraduates, graduate students, and postdoctoral fellows to conduct research in quantitative cardiovascular space physiology. In addition, the Center sponsors a number of educational activities to expose this field to students, university investigators, and scientists and engineers in government and industry.

Fiscal year 1997 has been productive for the Center. We continued to pursue a number of research directions relevant to improving our understanding of the effect of space flight on the cardiovascular system. These directions include cardiovascular system identification and related analyses of hemodynamic variability, development of T-wave alternans techniques for identification of individuals at risk for cardiac arrhythmias, and finite element modeling of cardiac electrical activity.

One of the major physiological concerns associated with space flight is cardiovascular deconditioning which manifests itself most notably as marked orthostatic hypotension upon return to Earth's gravity. The mechanisms underlying cardiovascular deconditioning remain unclear. We have developed new techniques for quantitatively studying alterations in cardiovascular regulatory mechanisms based solely on analysis of fluctuations in hemodynamic measurements. Hemodynamic values such as heart rate and blood pressure fluctuate on a beat-to-beat basis and these fluctuations reflect the responses of cardiovascular regulatory mechanisms to ongoing physiologic perturbations. We have developed new technologies based on system identification for quantitatively characterizing such important cardiovascular regulatory mechanisms as the heart rate baroreflex based solely on measurements of fluctuations in heart rate, blood pressure, and instantaneous lung volume. In 1997, we continued to publish results demonstrating the efficacy of these techniques. We used pharmacological autonomic blockade and posture changes to demonstrate that the coupling mechanisms characterized by our techniques respond appropriately to these known interventions [Mullen *et al.*, 1997]. We also applied these techniques to evaluate alterations in autonomic modulation of heart rate during moderate motion sickness [Mullen *et al.*, In Press] and during low dose administration of scopolamine [Raeder *et al.*, 1997]. We have continued to evaluate the use of both linear and nonlinear models. In 1997, we published a comparative review of representative techniques [Chon *et al.*, 1997], as well as a demonstration of neural network-based approaches for efficiently and accurately characterizing linear and nonlinear models [Chon *et al.*, 1997]. Finally, we also presented a new method for detection of chaotic determinism in time series such as heart rate [Chon *et al.*, 1997]. These technologies provide important new noninvasive approaches for the study of cardiovascular regulatory function which promise to provide important insight into alterations in cardiovascular function associated with exposure to microgravity. In 1997, we continued to improve the techniques and added measurement of cardiac output which enables us to investigate additional regulatory mechanisms such as the resistance baroreflex. We continue to evaluate these techniques in ground-based models in which cardiovascular regulatory dysfunction is either known or anticipated.

There is significant anecdotal evidence that exposure to microgravity may be associated with an increased incidence of ventricular arrhythmias. It would be desirable to quantitatively assess alterations in cardiac electrical stability and any related increase in susceptibility to ventricular arrhythmias associated with microgravity exposure. A new technology based on interpretation of T-wave alternans measured in surface electrocardiograms has proven to be an accurate predictor of increased susceptibility to ventricular arrhythmias. This technology was developed in the Center and has now been licensed to Cambridge Heart, Inc. It has received FDA clearance and is currently being evaluated in clinical studies worldwide. We published a review of the clinical utility of T-wave alternans [Armoundas *et al.*, 1997]. Accurate assessment of T-wave alternans requires that the heart rate be elevated so in the past, atrial pacing has been used. We published a report demonstrating that T-wave alternans may be reliably assessed when exercise stress is used instead of pacing [Hohnloser *et al.*, 1997]. We have also demonstrated that T-wave alternans evaluated during exercise stress is as effective as the invasive procedure of electrophysiological testing in identifying patients at increased risk for ventricular arrhythmias [Estes *et al.*, In Press]. These are extremely important results since they demonstrate that T-wave alternans can provide a completely noninvasive tool for the assessment of susceptibility to ventricular arrhythmias during real and simulated space flight.

Computational models are an important tool for expanding our understanding of the mechanisms which lead to alterations in cardiac electrical stability and an increased susceptibility to ventricular arrhythmias, particularly alterations associated with microgravity exposure. We have undertaken the development of computationally efficient and quantitatively reliable discrete element models of action potential propagation in myocardial tissue [Feldman *et al.*, In Press]. These models are highly desirable since arrhythmogenesis in tissue with altered electrical properties depends not only on the detailed changes in the membrane ionic currents, but on how these changes manifest themselves into macroscopic reentrant activity throughout the full 3D myocardial volume.

Simulation of electrical activity over such macroscopic scales is not possible using the standard reaction-diffusion partial differential equation (PDE) models of myocardial cells, since these models are very complex and only suitable for simulation over small (1 cm x 1cm) patches of 2D tissue. Clearly, these models cannot be used to perform large-scale simulations over different realizations (alterations) of the tissue for varying initial conditions, as is required for analysis of arrhythmogenesis. Moreover, specific features of propagating action potentials (such as the conduction velocity and refractory period) cannot be controlled independently, since the equations used are typically empirically derived, and the model parameters are dependent on one another. Our focus has been to extract from such models the characteristic features of excitation and recovery that control the behavior of the waves and incorporate them into our discrete element representation. We have demonstrated a quantitative correspondence between the traveling waves in our discrete system and those found in simple PDE models representing excitable tissues [Chernyak et al, 1997, Feldman *et al.*, 1997]. This correspondence allows us to reliably simulate macroscopic activity and study the potentially proarrhythmic effects of specific alterations in tissue properties that may arise in a zero-gravity environment.

The potential Earth benefits of the Center's research efforts are numerous. The system identification approaches to the analysis of hemodynamic variability promise to provide a new tool for evaluating the cardiovascular regulatory status of patients. Such a tool would be useful in diagnostic medicine as well as in the evaluation of therapeutic efforts. We have begun evaluating these techniques in a number of clinical applications such as in the diagnosis of early stage diabetic autonomic neuropathy and in assessing the hemodynamic status of patients with congestive heart failure. These techniques also provide a new research tool for advancing our understanding of integrated cardiovascular regulatory mechanisms.

The T-wave alternans technologies are now well recognized worldwide as a potential tool for identifying patients at risk for ventricular arrhythmias. Nearly 400,000 individuals a year die of sudden cardiac death in which the presumed cause of death is a fatal ventricular arrhythmia. Many of these individuals could be saved by antiarrhythmic agents or the implantation of a cardioverter/defibrillator if they could be identified prior to the fatal event. The T-wave alternans technology promises to provide the first noninvasive means for identifying such individuals.

The Center's cardiac electrical models provide a quantitative means of investigating cardiac conduction processes. The models promise to aid in the continued advancement of our understanding of clinically relevant cardiac conduction issues. They also promise to provide a means of predicting potential arrhythmic effects of various pathologies and for developing, evaluating, and testing of new antiarrhythmic therapies.

FY97 Publications, Presentations, and Other Accomplishments:

Armoundas, A.A. and Cohen, R.J. "Clinical utility of T-wave alternans." *Cardiac Electrophysiol. Rev.*, 3, 390-394 (1997).

Chernyak, Y.B., Feldman, A.B., and Cohen, R.J. "Correspondence between discrete and continuous models of excitable media: Trigger waves." *Physical Rev. E*, 55, 3215-3233 (1997).

Chon, K. and Cohen, R.J. "Linear and nonlinear ARMA model parameter estimation using an artificial neural network." *IEEE Transactions Biomed. Engin.*, 44, 168-174 (1997).

Chon, K., Kanters, J.K., Cohen, R.J., and Holstein-Rathlou, N.H. "Detection of chaotic determinism in time series from randomly forced maps." *Physica D*, 99, 471-486 (1997).

Chon, K., Mukkamala, R., Toska, K., Mullen, T.J., Armoundas, A.A., and Cohen, R.J. "Linear and nonlinear system identification of autonomic heart-rate modulation." *IEEE Engin. Med. Biol.*, 16, 96-105 (1997).

Estes, M.N.A., Zipes, D.P., El-Sherif, N., Venditti, F.J., Rosenbaum, D.S., Albrecht, P., Wang, P.J., Krause, P.C., and Cohen, R.J. *J. Am. Cardiol.* (In Press).

Feldman, A.B., Chernyak, Y.B., and Cohen, R.J. "Spiral waves are stable in discrete models of homogeneous two-dimensional excitable media." *Int. J. Bifurcation and Chaos in Appl. Sci. & Engin.* (In Press).

Feldman, A.B., Chernyak, Y.B., and Cohen, R.J. "Kinematic analysis of spiral waves in a discrete element model of neuromuscular tissue." *Proceedings of the International Conference of the IEEE EMBS Society*, 19:(Paper 19 CD Edition) (1997).

Hohnloser, S.H., Klingenhoben, T., Zabel, M., Li, Y.G., Albrecht, P., and Cohen, R.J. "T wave alternans during exercise and atrial pacing in humans." *J. Cardiovasc. Electrophysiol.*, 8, 987-993 (1997).

Mullen, T.J., Appel, M.L., Mukkamala, R., Mathias, J.M., and Cohen, R.J. "System identification of closed-loop cardiovascular control: Effects of posture and autonomic blockade." *Am. J. Physiol.*, 272, H448-H461 (1997).

Mullen, T.J., Berger, R.D., Oman, C.M., and Cohen, R.J. "Human heart rate variability relation is unchanged during motion sickness." *J. Vestib. Res.* (In Press).

Raeder, E.A., Stys, A., and Cohen, R.J. "Effect of low-dose scopolamine on autonomic control of the heart." *Ann. Noninvasive Electrocardiol.*, 2(3), 236-241 (1997).

Effects of Acute Intense Exercise and Microgravity on Mechanisms Associated with Blood Pressure Regulation in Humans

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-14-17-01

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Expiration: 1999

Students Funded Under Research: 4

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Task Description:

Reductions in plasma volume (hypovolemia relative to 1-G) and autonomic dysfunction in humans have been documented following exposure to actual and ground-based simulations of microgravity. Since these cardiovascular adaptations are associated with post-space flight orthostatic hypotension, partial or complete restoration of microgravity-induced alterations in vascular volume and autonomic functions should therefore enhance orthostatic stability and contribute to the safe return and rapid recovery of crew members. Cycle ergometry exercise designed to elicit maximal effort has been successfully used to increase plasma volume and carotid baroreflex sensitivity in ambulatory subjects. Therefore, the purpose of this investigation is to test the hypothesis that a single bout of cycle exercise designed to restore plasma volume and reverse autonomic dysfunction within 24 hr of reambulation following exposure to simulated microgravity can ameliorate orthostatic hypotension and intolerance. A three-year human physiology research project is presented in this proposal which is designed to: (a) describe dynamic changes in blood volume, hormone responses, autonomic functions, and hemodynamic responses to orthostatic hypotension induced by exposure to an analog of microgravity; (b) describe interactions of these systems with each other; and (c) test the responses of these systems during the 24-h recovery period following acute intense exercise. The study will be conducted using 16 days of 6° head-down tilt (HDT) to determine the effects of extended duration exposure to microgravity on mechanisms that contribute to blood pressure control and if the restoration of these mechanisms will reverse orthostatic intolerance. Plasma volume, leg compliance cardiopulmonary and arterial baroreflex functions, adrenoreceptor function, cardiac and hemodynamic measurements, and vasoactive hormone responses will be measured in subjects before and after HDT with and without exercise treatment to determine the effect of reversing altered mechanisms associated with blood pressure regulation on orthostatic tolerance. Our expected result from this investigation is that a single exposure to acute exercise designed to elicit maximal effort within 24 hr of reambulation from HDT will provide a stimulus that reverses hypovolemia and autonomic dysfunctions induced by microgravity and eliminate orthostatic intolerance. Results of these studies should provide a better understanding of the adaptive process of components of the blood pressure control system during recovery from acute exercise and to microgravity environments, and a physiological basis for development of specific effective countermeasures against orthostatic hypotension following space flight.

In addition to the publications listed in the bibliography, we completed an additional eight manuscripts and one book chapter that are currently in press. Based on the results from our completed experiments, we continue to write manuscripts describing hemodynamic adaptations and mechanisms of fluid homeostasis during exposure to microgravity, and development of exercise countermeasures. In addition, we have proceeded with preliminary work to extend our experiments during FY98 to investigate the effects of acute maximal exercise in ambulatory human subjects on central venous pressure setpoint, cardiac vagal tone, cardiopulmonary baroreflex control of vascular resistance, and aortic baroreflex control of heart rate. We requested and received approval from NASA headquarters for a no-cost extension to our grant since major reorganization of the Air Force Research Lab has limited our ability to complete the funded research by the end of FY97 or FY98.

Results from our experiments should provide new understanding of mechanisms underlying the clinical condition of orthostatic hypotension, from patients who are restricted to prolonged bedrest or with autonomic dysfunctions to astronauts following a space mission. The results from the testing of acute intense exercise proposed in this research can provide a new potential therapeutic for acute management of orthostatic hypotension and intolerance. We have already implemented the use of this protocol to eliminate orthostatic hypotension in a group of paraplegic patients. The results of this research could provide a simple technique to help alleviate clinical symptoms associated with orthostatic hypotension.

FY97 Publications, Presentations, and Other Accomplishments:

Buckworth, J., Convertino, V.A., Cureton, K.J., and Dishman, R.K. "Increased finger arterial blood pressure after exercise detraining in women with parental hypertension: Autonomic tasks." *Acta Physiol. Scand.*, 160, 29-42 (1997).

Buckworth, J., Cureton, K.J., Convertino, V.A., Jacobs, K., and Dishman, R.K. "Orthostatic responses in women with parental hypertension: Fitness and carotid baroreflex function." (Abstract) *Med. Sci. Sports Exercise*, 29, S89 (1997).

Convertino, V.A. "Autonomic disorders associated with conditions of reduced gravity" in "Clinical Autonomic Disorders." Edited by: Low, P.A., 2nd ed., Chapter 34. Boston: Little, Brown and Co., pp 429-440 (1997).

Convertino, V.A. "Cardiovascular consequences of bedrest: Effects on maximal oxygen uptake." *Med. Sci. Sports Exercise*, 29, 191-196 (1997).

Convertino, V.A. and Reister, C.A. "Effect of G-Suit protection on carotid-cardiac baroreflex function." (Abstract) *Aviat. Space Environ. Med.*, 68, 658 (1997).

Convertino, V.A., Bloomfield, S.A., and Greenleaf, J.E. "An overview of the issues: Physiological effects of bedrest and restricted physical activity." *Med. Sci. Sports Exercise*, 29, 187-190 (1997).

Convertino, V.A., Polet, J.L., Engelke, K.A., Hoffler, G.W., Lane, L.D., and Blomqvist, C.G. "Evidence for increased b-adrenoreceptor responsiveness induced by 14 days of simulated microgravity in humans." *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)*, 273, R-93-R-99 (1997).

Convertino, V.A., Previc, F.H., Ludwig, D.A., and Engelken, E.J. "Effects of vestibular-oculomotor stimulation on responsiveness of the carotid-cardiac baroreflex." *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)*, 273, R615-R622 (1997).

Evaluation of the Hemodynamic Mechanism Underlying Cardiovascular Adaptation in a Chronically Instrumented Rhesus Model During Simulated Microgravity

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Funding:

UPN/Project Identification: 199-14-17-07	Solicitation: not available
Initial Funding Date: 1994	Expiration: 1997
Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: not available	
Joint Agency Participation: DoD (USAF)	

Task Description:

An operational problem for astronauts is the compromised regulation of blood pressure associated with their removal from gravity stimulus that may result in orthostatic intolerance, attenuated adrenergic responsiveness, and physiological deconditioning. A decrease in central venous pressure (CVP) despite maintained or increased cardiac output has been observed during space flight and in ground-based bedrest studies. The primary objective of this research is to invasively measure specific hemodynamic responses in a non-human primate model during exposure to 10 degrees head-down tilt (HDT), a surrogate of microgravity, in order to test two hypotheses that may explain mechanism(s) of decreased CVP in the face of maintained/increased cardiac output caused by space flight. These hypotheses are: 1) that there is an increase in cardiac compliance associated with exposure to microgravity, and/or 2) that there is a resetting of the CVP set-point to a lower operating range. Hemodynamic and adrenergic data will be obtained from ten chronically-instrumented rhesus monkeys. The test protocol consists of five days exposure to 10 degree head-down tilt (treatment condition) and five days of 80 degree head-up tilt (control condition) separated by one week of return to baseline in a cross-over counterbalance design. Hemodynamic measurements will include pressures of the left ventricular, right atrium, aorta, and esophagus, aortic flow, cardiac output, cardiac chamber areas (transesophageal echocardiography), hormone levels, and plasma volume. Provocative test measurements will include Dextran infusion, phenylephrine infusion

(alpha-receptor sensitivity), isoproterenol infusion (beta-receptor sensitivity), and lower body positive and negative pressure. Identifying mechanisms underlying the reduction in CVP in microgravity could prove instrumental to the development of effective countermeasures against orthostatic hypotension induced by both G-layoff or space flight.

We completed a manuscript entitled "Evidence for increased compliance during exposure to simulated microgravity" and submitted it for review and publication in the *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)*. Based on the results from our completed experiments, we continue to write manuscripts describing models for hemodynamic adaptations to microgravity and mechanisms of fluid homeostasis during exposure to HDT. In addition, we have proceeded with preliminary work to extend our experiments during FY98 to investigate mechanisms involved in fluid homeostasis during spaceflight. We plan to use our HDT model to expose rhesus monkeys to the following two 3-day experimental conditions separated by 11 days of return to baseline ambulatory activities in a cross-over counterbalance design: 1) continuous exposure to 10° HDT; and 2) 16 hours per day of 80° head-up tilt and 8 hours supine (control). Each animal will undergo measurement of renal function during fluid loading with and without aldosterone infusion to determine if possible changes in renal tubular mechanisms associated with body fluid homeostasis occur during exposure to a ground-based analog of microgravity. We requested and received approval from NASA headquarters for a no-cost extension to our grant since major reorganization of the Air Force Research Lab has limited our ability to complete the funded research by the end of FY97 or FY98.

Results from these experiments should provide new understanding of mechanisms underlying the regulation of plasma volume and CVP during conditions of physical deconditioning or restricted bedrest. This knowledge could be instrumental in the development of therapeutic management for dehydration effects in patients with restricted physical activity as well as with astronauts following a space mission. These mechanisms could contribute to the orthostatic hypotension and intolerance experienced by both patients and astronauts. Our results will also provide some new insight into the cardiovascular effects of ketamine, a human pediatric anesthetic. If reduced CVP setpoint proves to be an adaptation in these experiments, this could provide a basis for development of new therapeutic techniques designed to acutely increase the CVP setpoint to enhance vascular volume, cardiac filling pressure, and consequently, defend blood pressure regulation during orthostatic challenges.

FY97 Publications, Presentations, and Other Accomplishments:

Convertino, V.A., Koenig, S.C., Krotov, V.P., Fanton, J.W., Korolkov, V.I., Trambovetsky, E.V., Ewert, D.L., Truzhennikov, A., and Latham, R.D. "Effects of 12 days exposure to simulated microgravity on central circulatory hemodynamics in the rhesus monkey." *Acta. Astronom.* (In Press).

Krotov, V.P., Convertino, V.A., Korolkov, V.I., Latham, R., Trambovetsky, E.V., Fanton, J., Crisman, R., Truzhennikov, A.N., Ewert, D., Nosovsky, A.M., and Conolly, J. "The heart function during two weeks of antiorthostatic hypokinesia." *Sechonov Physiol. J.*, 82, 34-45 (1996).

Blood Volume Regulation in Primates During Space Flight

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UPN/Project Identification: 199-14-17-12

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Expiration: 1997

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

During space flight, it has been observed that astronauts become hypovolemic and undergo some cardiac deconditioning. It has been documented that there is a central shift of both blood and tissue fluids resulting in an increase in central venous pressures. It is assumed that this fluid shift activated the Henry-Gauer reflex, producing a diuresis and natriuresis. Unfortunately, an immediate diuresis associated with the insertion into orbit has not been well documented.

The objectives of this study are to examine the renal and cardiovascular responses of the primate to weightlessness, determine if there is an immediate diuresis and natriuresis, and determine if this response contributes to the cardiac deconditioning. Rhesus monkeys will be instrumented with aortic, left atrial, and superior vena caval catheters, and aortic, carotid, iliac, and renal blood flow probes. We will study the alterations in the renal responses to increases in central blood volume to determine if there are changes in the reflex control of blood volume during prolonged exposure to weightlessness. Finally, we will examine the effects of chronically increasing blood volume with salt loading on the control of blood volume and blood pressure in simulated and space flight conditions.

Our objective is to develop a ground-based model which will allow us to study the control of blood volume in the primate under simulated weightlessness conditions. Animals will be subjected to partial immersion for 72 hours while repeating those studies conducted in space. In addition, the effects of chronic salt loading on the reflex control of blood volume and blood pressure during prolonged immersion will be examined.

While the design and objectives of this study are relatively straightforward, their implementation is very time intensive and fraught with the peculiar challenges associated with conscious monkeys. For these reasons this protocol was not designed to collect massive amounts of data but rather to produce a primate model of microgravity that would allow us to address some of the specific problems associated with the adaptation of the primate to the microgravity environment. The individual objectives were to:

- 1) Develop a non-human primate model that simulates the cardiovascular deconditioning observed after exposure to the mG environment.

- 2) Determine the progressive alterations in the baroreflex control of blood pressure during and after 72 hrs of exposure to simulated microgravity.
- 3) Determine if there is an alteration in the control of blood volume during simulated microgravity.
- 4) Investigate possible countermeasures that could be used to prevent the orthostatic hypotension that has been observed in the astronauts.

Eight chronically instrumented Rhesus monkeys were used in this study. Cardiovascular instrumentation included arterial and venous catheters and aortic and renal Doppler flow probes. After training the animals to the restraint chair, they were placed in a water-tight suit and immersed in the upright position to the level of the mid chest. The immersion period lasted for 72 hrs and was preceded and followed by two hours of control. Renal and cardiovascular data were recorded continually and urine was collected hourly. The psychological well-being of the animal was ensured by having someone with them continually as well as providing them with soft music and a variety of fruits. The animals tolerated this very well, ate and drank during the procedure, and interacted with the technician.

During the control immersions, no supplemental volume was provided. Catheter lines were maintained patent by infusing lactated Ringer's solution at a rate of 3 ml/hr/catheter. The baroreflex was determined before immersion, 3 hours into the immersion, at 22, 46, and 70 hrs of immersion and then after the immersion. The first intervention was to maintain fluid balance by infusing 51 ml lactated Ringer's solution/hr over the entire study period. Alterations in the control of blood volume were determined by volume expanding the animals with 6% dextran in normal saline (this is isotonic isoncotic). The control volume expansion was done on a day other than the immersion day and on the last day of the immersion. This was done with and without the maintenance of fluid volume.

Results:

Objective 1: Develop a non-human primate model that simulates the cardiovascular deconditioning observed after exposure to the microgravity environment.

- 1) There was an increase in blood pressure and CVP during the immersion which gradually return towards control by the end of the immersion. Blood pressure and CVP returned to or below controls when the monkey was removed from the tank. This was associated with a decreases in heart rate during the immersion. After the immersion the heart rate was significantly elevated above pre-immersion levels.
- 2) Seventy two hours of immersion caused the baroreflex curve (HR vs BP) to shift upwards and to the right. This was associated with a decrease in the slope of the curve.
- 3) The animals become slightly hypotensive during the first hr after immersion.
- 4) A significant negative water balance began within 6 hrs of the immersion and gradually progressed throughout the immersion period, even though the animal has free access to water. Most of this water imbalance was due to a decrease in water intake, although there was also a significant increase in urine flow during the immersion.
- 5) ANF initially increased early in the immersion and then returned to or below normal within 24 hrs of immersion. After the immersion there was an increase in ANF, probably due to the baroreflex tachycardia.
- 6) During the immersion there was a significant diuresis that began gradually and was maintain throughout the period of immersion. This was associated with a similar increase in sodium excretion.
- 7) After the immersion, the blood pressure was very sensitive to the administration of nitroprusside and relatively insensitive to phenylephrine.

Objective 2: Determine the progressive alterations in the baroreflex control of blood pressure during and after 72 hrs of exposure to simulated mG.

Six baroreflex curves (HR vs BP) were determined during each experiment, one before and after the 72 hrs of immersion, one 3 hrs into the immersion and then at 22 hrs, 46 hrs and 70 hrs of immersion. After the immersion there was a significant decrease in the sensitivity of the baroreflex control of heart rate associated with alteration in blood pressure. Initially (3 hrs), the baroreflex curve shifts downward and to the left. This

was primarily a decrease in the tachycardic portion of the curve resulting in a decreased sensitivity. After 22 hrs of immersion the curve shifted back towards the normal curve. However, the resting position along the curve shifted to the right along the blood pressure axis. After immersion the curve shifted upwards and to the right, resulting in a significantly increased resting heart rate at a normal or decreased blood pressure. There was also a significant decrease in the slope of the curve.

Objective 3: Determine if there is an alteration in the control of blood volume during simulated microgravity

Volume expansion during immersion without supplemental volume infusion.

This intervention was intended to determine the sensitivity of the volume control mechanisms. It also provided information on the effects of filling the vascular space on the control of blood pressure post immersion.

- 1) A volume expansion with isoncotic isotonic dextran during the last 6 hours of immersion did not cause a significant diuresis.
- 2) The baroreflex curve was shifted upwards with the same saturation. However there was still tachycardia post immersion as seen in the control immersions.
- 3) The animals did not become hypotensive to any degree during post immersion period. They were also less sensitive to nitroprusside and more responsive to phenylephrine.
- 4) The animals were still in a negative water balance at the end of the immersion.

Volume expansion with supplemental volume infusion.

The volume expansion was intended to test the reflex control of blood volume during the immersion. It also replenished both the depleted vascular volume as well as the extracellular volume that had been lost during the immersion.

- 1) There was a significant diuresis in response to the dextran infusion given just before de-immersion. In some instances, this exceeded the volume given during the expansion.
- 2) The baroreflex curve still shifts upwards and to the right after the immersion.
- 3) There is a decreased tachycardia post immersion.
- 4) Some of the animals were in a negative fluid balance post immersion.
- 5) The animals were relatively insensitive to nitroprusside post immersion and very sensitive to phenylephrine.

Objective 4: Investigate possible countermeasures that could be used in order to prevent the orthostatic hypotension that has been observed in astronauts.

Immersion with maintenance fluid volume.

In this protocol the hydration level of the animal was established for 3 hours before the immersion by infusing 50 ml/min lactated Ringer's solution. This was continued throughout the immersion. This intervention expanded both the vascular and extravascular spaces.

- 1) The CVP and blood pressure increases more during the immersion than in the control experiments. Heart rate was also significantly lower during the immersion than in the control studies. After immersion, the blood pressure tended to be at or above the pre-immersion control levels while the heart rate was less.
- 2) The baroreflex still shifted to the right, however, the slope did not change. There was a decreased hypotension with nitroprusside. The animals did not become hypotensive during the post immersion period.
- 3) The animals still went into a negative water balance, however, not to the same degree as without the infusion. The negative water balance was due almost completely to adipsia in the animals even though they continued to eat normally.
- 4) The alterations in ANF were similar to the control condition.
- 5) The diuresis and natriuresis were greater and were maintained throughout the immersion.

Conclusions:

Water immersion of the non-human primate to the level of the mid-chest simulates the cardiovascular changes reported during microgravity in astronauts. Our results would suggest that there is a diuresis during exposure to microgravity related to the level of hydration. This diuresis would probably be most evident early in the flight. Volume maintenance before and during microgravity may actually increase the diuresis while decreasing the degree of post flight orthostatic intolerance. It would appear that the control of blood volume is enhanced during microgravity. Therefore, volume loading prior to re-entry may enhance the diuresis associated with increases in volume intake. Fluid loading during microgravity would decrease the sensitivity to hypotensive events by restoring vascular and extra vascular volumes and maintaining the sensitivity of the baroreflex. However, the baroreflex would still be reset at an increased heart rate after exposure to the microgravity environment.

There are several conditions which cause alterations in the control of blood pressure and fluid volume. The changes reported here in alterations associated with the baroreflex during simulated microgravity are similar to those reported with chronic congestive heart failure. However the alterations observed in the reflex control of blood volume are not consistent with those seen in heart failure. It may be that there are similarities with other disease conditions that represent states of relative hypovolemia.

In many regards the observation of post flight orthostatic intolerance are similar to the orthostatic intolerance reported in man. Under these conditions, there appears to be both a volume component and a cardiovascular reflex component. Most of the interventions are directed at the changed reflex component. This study would suggest that adequate volume control may prevent the severity of the orthostatic hypotension even though it doesn't restore the altered reflex component.

Autogenic Feedback Training as a Preventive Method for Orthostatic Intolerance

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Funding:

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Post-Doctoral Associates: 2

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Responsible NASA Center: Ames Research Center

Task Description:

Post-flight orthostatic hypotension has been identified as a serious biomedical problem associated with sustained exposure to micro-G. The general purpose of this research is: (1) to learn the most effective ways of training subjects to produce large, voluntary increases in blood pressure; (2) to determine the effectiveness of this training at counteracting various conditions producing orthostatic hypotension; (3) to understand the cardiovascular, endocrine, and other mechanisms involved; and (4) to determine if certain of the mechanisms discovered to be most prominently involved can be used to produce more effective training.

The first study is entitled: "A comparison of blood pressure feedback training alone vs. multiple response feedback training: Effects on orthostatic intolerance." This study will also determine if there are differences in the training results of subjects with initially high or low orthostatic tolerance. Forty-eight men and women will be assigned to three groups (N = 16), matched for orthostatic tolerance (eight low- and eight high-tolerance subjects per group) during initial presyncopal lower body negative pressure (LBNP) tests. Groups are Multiple Response Feedback, Blood Pressure Feedback only, and No Treatment Control.

Progress during FY97: A study was completed entitled "A Comparison of Supine Lower Body Negative Pressure to Combined Head-Up-Tilt and LBNP for Inducing Presyncope in Men."

The primary purpose of the present study was to directly compare two tests of orthostatic tolerance in normal adult men. The first was a supine LBNP test and the second was a combined test of head-up tilt (HUT) and LBNP. In order to test countermeasures for post-flight orthostatic intolerance, it is necessary that investigators understand the nature of physiological responses to a gravitational stress. We believe that each individual will

produce a unique physiological response pattern that will reliably describe his/her own symptom levels. We will determine which of these types of tolerance test is best suited for subsequently evaluating treatments or countermeasures which will be used to help future astronauts adapt more readily to microgravity as well as facilitate readaptation to Earth. Eight normal men participated in this study. Average age 37.5 ± 8.52 (mean \pm SD); they weighed 89.5 ± 8.9 kg and were 180.97 ± 5.2 cm tall. Data were collected using hardware and software developed under this task: The Ames Autogenic-Feedback Training System (see U.S. Patent #5,694,939), which enabled continuous measurement and display (on-line) of 21 different physiological parameters, as well as real-time calculations of indices of cardiovascular dynamics. The data successfully collected included: (1) electrocardiography; (2) respiration rate; (3-5) pulse volume (photoplethysmography) measured on left and right hands and right toe; (6) skin temperature; (7) skin conductance level; (8-10) blood pressure (systolic, diastolic, and mean arterial pressure measured beat-to-beat); (11-14) electromyography (measured at 4 sites, forearm extensors and gastrocnemius); (15) thoracic fluid index (volume); (16) stroke volume; (17) cardiac output; (18) total peripheral resistance; (19) estimate of vagal tone; (20) cerebral oxygenation; and (21) intracranial pressure. Results showed that subjects could tolerate the supine LBNP significantly longer than the combined HUT+LBNP ($p < 0.0004$). Analyses of physiological data are in progress. Conclusion: The combined stimulus (HUT+LBNP) reliably produces presyncope in all test subjects and will be used as the primary stimulus in blood pressure conditioning experiments as a means of assessing training effectiveness for preventing orthostatic intolerance. All data acquisition systems provide reliable indices of stimulus intensity and the pre-syncope endpoint.

Note: The above study was not part of the peer-reviewed, approved proposal. Investigators were required by NASA HQ to conduct this study before proceeding with planned experiments on training control of blood pressure. The blood pressure study as proposed, is now in progress.

AFTE is a method for training human subjects to voluntarily control several of their own physiological responses within a six-hour instruction program. The primary uses of this treatment are: (1) to facilitate adaptation to environmental stressors; (2) improve operator performance; and (3) correct disturbances in autonomic function. AFT has been tested during shuttle missions as a treatment for space motion sickness, during ground-based tests for terrestrial motion sickness, and in high-performance military aircraft for air-sickness. Additional applications include improved pilot performance during emergency search and rescue conditions, as a countermeasure for orthostatic intolerance in aerospace crews, and as a treatment for clinical patients suffering from hypotension, hypertension, nausea resulting from chemotherapy, and other disorders related to autonomic dysfunction. AFT can also be used to modify central nervous system (CNS) activity in the treatment of neuropathological disorders such as epilepsy, attention deficit disorder, and mild head trauma. Neurofeedback training has been used to alter brain activity resulting in the ability to modify effects of sleep deprivation on cognitive performance, and to facilitate sleep by reducing disturbances in circadian rhythmicity. Specific examples of application of AFTE benefits for Earth currently being investigated are:

- 1) Space Act Agreement in progress with University of Tennessee: AFTE as a potential treatment for Chronic Intestinal Pseudo-Obstruction Syndrome (autonomic neuropathy, symptoms of vomiting, nausea, and syncope). This technology has been shown to significantly reduce symptoms in 7 patients and may lead to a new treatment regime for dysautonomia world-wide. Requests from other national and international laboratories have been received for transfer of this technology.
- 2) Space Act Agreement in progress with University of Pennsylvania: AFTE as a potential treatment for Meniere's Disease symptoms of nausea and syncope.
3. Interagency Agreement completed with U.S. Army Tank-automotive and Armament Command: to evaluate the incidence and frequency of motion sickness episodes in the Command and Control vehicle using ambulatory monitor equipment designed to monitor autonomic function of crew members in space. Results: This technology can be used to reliably assess the impact of environmental stressors on human physiology and performance during field conditions. Therefore, this study stands as a model for use of this assessment technique under conditions of long-term space flight.

4. Interagency Agreement initiated between NASA Ames Research Center, Moffett Field, California and the US Army 16th Armored Cavalry Regiment, Ft. Knox, Kentucky: Autogenic-Feedback Training Exercise to Improve Performance During Armor Officer Basic Training.

5. Interagency Agreement initiated between NASA Ames Research Center, Moffett Field, California and the US Army Tripler Medical Center, Honolulu, Hawaii: Development of Telemedicine Capability for Remote Application of Autogenic-Feedback Training Exercise.

FY97 Publications, Presentations, and Other Accomplishments:

Patent Pending, U. S. Patent #: 5,694,939. Cowings, P.S. "Autogenic-Feedback Training Exercise (AFTE) Method and System."

Cowings, P.S. and Toscano, W.B. "Monitoring and correcting human autonomic function during long-term adaptation to microgravity." Presentation at the annual meeting of the American Autonomic Society and Clinical Autonomic Research Society, Toronto (October, 1996).

Cowings, P.S., Stout, C., Toscano, W.B., Reynoso, S., DeRoshia, C., Miller, N.E. The effects of promethazine on human performance, mood states and motion sickness tolerance. NASA Tech Brief #110420 (November, 1996).

Blood Pressure - Determinants and Controllers

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 5 P01 HL29587-15 (NIH) Solicitation: not available
Initial Funding Date: 1992 Expiration: 1998
Students Funded Under Research: 0 Post-Doctoral Associates: 0
FY 1997 Funding: \$62,499
Joint Agency Participation: NIH/National Heart, Lung, and Blood Institute

Task Description:

This task represents a supplement to a larger NHLB project. Additional information on the component funded by NASA was not available in time for publication.

Evaluation of Intermittent Bright Light Exposure as a Space Flight Countermeasure

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Funding:

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Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

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Students Funded Under Research: 1

Post-Doctoral Associates: 2

FY 1997 Funding: \$127,409

Task Description:

In order to enable astronauts to sustain high levels of performance throughout extended duration space missions it will be critical (1) to maintain an appropriate phase relation of the human circadian pacemaker to the 24-hour sleep-wake/duty schedule aboard the space station, and (2) to preserve the amplitude of the endogenous circadian timing system. On Earth, the amplitude of the endogenous circadian pacemaker and the phase relationship between the circadian pacemaker and the sleep-wake/duty schedule are preserved by exposure to the 24-hour, robust cycle of light and darkness associated with the Earth's rotation. During extended duration space station missions, astronauts will be exposed to markedly abnormal light/dark cycles in terms of both timing and intensity. Preliminary data suggest that such abnormal light/dark cycles are likely to result in a misalignment between the 24-h sleep-wake/duty schedules and the endogenous circadian timing system. Such circadian misalignment is known to produce sleep disturbances, daytime sleepiness, reduced attention, negative mood, slower reaction times, gastrointestinal disorders, and impaired daytime alertness. To prevent such misalignment, development of effective countermeasures to promote circadian entrainment aboard the space station is needed. Scheduled exposure to 5-h episodes of bright light has been shown previously to induce the necessary phase shifts and amplitude enhancement of the endogenous circadian pacemaker. However, energy and time constraints make such lengthy exposures to bright light impractical aboard the space station. Recent progress derived from experiments supported by our current NASA grant (NAGW-4033) indicates that three days of exposure to short, intermittent pulses of bright light are 2-3 times more effective in phase shifting the human circadian system than the continuous bright light exposures previously studied. However, our preliminary data do not allow us to estimate the effectiveness of a single sequence of intermittent light pulses, as we have previously employed three consecutive days of exposure to an intermittent stimulus. Therefore, on the basis of our preliminary data, we propose in this renewal application three testable hypotheses, the evaluation of which will be critical for the development of effective and attainable countermeasures. The hypotheses we plan to test are: (1) a single sequence of six pulses of intermittent bright light exposure (15 minutes light/1 hour dark) in one day will induce a significant phase delay of the endogenous circadian pacemaker; (2) the phase-shifting response per minute of

intermittent bright light exposure will be 2.5 times greater than that of continuous bright light exposure; and (3) endogenous circadian rhythms of body temperature, melatonin, cortisol, alertness, and performance will phase advance shift by an equivalent amount in response to a single, 6.5 hour episode of intermittent bright light exposure centered 3.5 hours before the temperature nadir.

Execution of the proposed studies will allow refinement of our current model of the phase shifting effect of light, allowing estimation of the optimal duration of bright light needed for a maximal phase shifting efficiency. This would lead to the development of a practical and efficient countermeasure to help maintain internal synchrony and prevent disruption of the circadian system. The results of the proposed research could thus have a profound effect on the health, productivity, and safety of astronauts during extended duration space station missions.

Based on new data from our laboratory on the dynamics of photic stimulation on melatonin suppression (Brown et al., 1997; *Am. J. Physiol.* 35) and phase shifting the clock (Kronauer et al., 1997; International Congress on Chronobiology, September 7 - 11; Paris, France), the stimulus has been moved such that it is centered 3.5 hours before the minimum rather than being centered 3.5 hours after the minimum of the fitted core body temperature rhythm. Based on earlier data published by our lab (Czeisler et al, 1989; *Science* 244), we anticipate robust phase delays upon exposure to the 6.5 hour continuous bright light stimulus, centered 3.5 hours before the minimum. Given this and earlier experimentation funded by NASA, we expect that delivering six 15 minute pulses of bright light, each pulse being separated by one hour of darkness, centered 3.5 hours before the minimum will also generate phase delays of a lesser magnitude than the continuous light, but the discontinuous light is hypothesized to be 2.5 times more effective per photon. An important consequence of changing the timing of the light stimulation is that it will allow us to examine the complex interaction among bright light, melatonin, and melatonin suppression, and its effects on EEG activity and performance. This is critical in the design of light stimulation during long-duration space missions due to the two-fold effects of light on the circadian system and the immediate effects on performance.

A greater understanding of the effects of brief episodes of light on the human circadian timing system will allow us to design shifting strategies which most efficiently utilize bright light exposure. These strategies are critical in situations in which circadian misalignment is problematic, such as long-duration space missions and shift work. During long-duration space missions, astronauts are exposed to a low-intensity light/dark cycle. Such exposure may result in a loss of synchronization between the internal circadian clock and the external light/dark cycle. This type of misalignment can result in problems in the performance, health, and safety of astronauts. By using brief episodes of light exposure, we will be able to prevent such misalignment from occurring. Our results will enable us to determine the most effective strategy possible to prevent such misalignment. Circadian misalignment can also occur in shiftworkers here on Earth, who are exposed to unusual patterns of light and dark due to the nature of their jobs. Utilizing maximally effective pulses of light will help us keep the internal rhythms of shiftworkers better aligned with that of their sleep/wake schedule. Not only does our research have immediate implications for both astronauts and shiftworkers, it also addresses some very basic questions about the human circadian timing system. We will use our results to further refine a model describing the characteristics of transduction of photic information in the human circadian timing system. This is useful not only in its descriptive power, but also in that it can help to identify the anatomical elements which may be involved in the processing of light information to the human circadian pacemaker.

FY97 Publications, Presentations, and Other Accomplishments:

Kronauer, R.E., Jewett, M.E., and Czeisler, C.A. "Modeling human circadian phase and amplitude resetting." International Congress on Chronobiology, Paris, France (September 7 - 11, 1997).

Shanahan, T.L., Zeitzer, J.M., and Czeisler, C.A. "Resetting the melatonin rhythm with light in humans." *J. Biol. Rhythms*, 12, 556-567 (1997).

Zeitzer, J.M., Kronauer, R.E., and Czeisler, C.A. "Photopic transduction implicated in human circadian entrainment." *Neurosci. Letters*, 232, 135-138 (1997).

Pre-Launch Adaptation of Orbiter Crew Members to Earlier Shifts Following Exposure to a Single Bright Light Episode: Clinical Trial Comparing the Response in Men to that in Women

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Funding:

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Students Funded Under Research: 1	Post-Doctoral Associates: 2
FY 1997 Funding: \$0	
Joint Agency Participation: NIH/National Institute of Mental Health	

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

It has been widely reported in many organisms that after exposure to a phase resetting stimulus, phase markers of the circadian system often display several cycles of non-steady-state behavior (referred to as 'transients') prior to reaching a stable phase position. It has been proposed that such transients may not reflect the time course of the phase shift of the underlying pacemaker but instead may reflect the time it took for a given phase marker to realign with the shifted pacemaker. This hypothesis has been tested in several organisms using a double-pulse experiment in which the phase response curve to two stimuli applied several hours apart was compared to the steady-state phase response curve to a single light stimulus. The results in several studies have suggested that the underlying pacemaker has in fact shifted rapidly to its final phase, and therefore that the transient behavior is a property of the phase marker which may be acting as a slave oscillator driven by the circadian pacemaker.

Although the phase shifting response to bright light stimuli has been explored in human subjects using a number of different circadian markers, the existence of potential transients in phase shifts of these markers has not been systematically studied. Therefore, in order to determine whether the core body temperature phase marker may exhibit transient phase shifts in humans, phase shifts to *steady-state* trials using a 3 cycle bright light stimulus (in which the initial circadian phase is known to be stable) were compared to those of *consecutive* trials (where an identical light stimulus was applied immediately following a prior *steady-state* trial). If the phase marker were to exhibit transients, then for any given phase of stimulus application, the *consecutive* trials would induce different apparent phase shifts in the marker than would be the case for *steady-state* trials at the same circadian phase.

The in-laboratory experimental protocol consists of 16 days in time isolation where the lighting conditions and the timing of the sleep-wake schedule are controlled by the investigators. Prior to entry into the study subjects are instructed to maintain a standard sleep wake schedule for 3 weeks in order to allow the pacemaker to come

into a steady state condition. Additionally, subjects are free from medical, psychiatric, and sleep disorders, are instructed to abstain from caffeine, nicotine, alcohol, and drugs for 3 weeks before their study, and are verified to be drug-free at the time of study. All subjects must deny a history of night work or shift work in the 3 years prior to study, and the crossing of more than 2 time zones in the previous 3 months prior to study. Following 3 baseline days in the laboratory, subjects then undergo a constant routine procedure designed to accurately assess the circadian phase of the core body temperature by eliminating the masking effects of sleep, posture, and other behaviors. On the next 3 consecutive days the sleep wake schedule are altered to accommodate a 5 hour bright light stimulus at a designated circadian phase in the middle of their waking episode. This is in turn followed by a second constant routine, another 3 days of bright light exposure, and finally by a third constant routine. The phase difference measured between the first and second constant routines represents the phase shift to light under steady state conditions (steady state trials). The phase difference between the second and final constant routines represents the phase shift to the second bright light stimulus (consecutive trials). If the phase shifts to consecutive trials are consistent with those of steady state trials, this would indicate that there is no transient behavior of phase shifts to these light stimuli.

In addition, in 1993, we proposed to carry out studies on the effect of intermittent light versus continuous light exposure on resetting the human circadian pacemaker. Preliminary data have revealed that 87.7% and 63.1% of the resetting response was preserved even when the bright light stimulus was interrupted with uniformly spaced intervals of complete darkness for 27% and 69% of the time, respectively. New data indicate that the maximal phase advance for a weak type 1 resetting occurs when the stimulus is centered later than the initial phase of administration used in these preliminary studies. Therefore, additional data have been collected by centering the light exposure 3.5-h after the initial fitted temperature minimum.

Efforts on this project over the course of the last fiscal year were targeted at determining whether there is any transient behavior in the phase shifts to a 3 cycle 5 hour bright light stimulus as measured by the circadian rhythm in core body temperature rhythm in human subjects. The in-laboratory experimental protocol used was the 16 day time described above.

Core body temperature was collected throughout all studies at 1 min intervals from a rectal thermistor. During the constant routines, subjects were kept awake in a constant semi-recumbent posture in dim light (10-15 lux) with minimal physical activity allowed, and food distributed in hourly snacks across the 24 h day. Phase and amplitude of the core body temperature data were estimated by fitting to the data from the constant routines a two-harmonic-regression model with first-order auto-regressive noise. The period of the fundamental component of the model was constrained between 24.0 and 24.3h. Phase (referred to as the estimated circadian phase of the core body temperature minimum) was defined to be the average of the phases of the minima from the single-harmonic and composite waveforms of the fitted model, and amplitude was defined as half the distance between the maximum and minimum of the first harmonic component of the model.

Phase shifts for steady state trials were defined to be the difference in clock time between the estimated minimum of the core body temperature rhythm in the first constant routine and the estimated minimum of the core body temperature rhythm in the second constant routine. For consecutive trials the phase shifts were the difference between the second and third constant routines. Data from all trials were plotted in phase response curve format (initial phase vs. phase shift). To determine the slope of the phase response curve, linear regressions were fit to the data. To evaluate transients, a linear regression fit to the *consecutive* trial phase response curve was compared to a linear regression fit to an similar portion of the *steady-state* trial phase response curve determined from data previously acquired in the laboratory using an identical steady state trial protocol. Subject's t-tests were used to compare the slopes and intercepts of the two regression lines.

A total of 7 subjects completed the 16 day protocol during the last fiscal year. Data from these subjects was supplemented with data from 1 subject who had completed a similar protocol prior to the last fiscal year. In the steady trials for these subjects, the 3 cycle light stimuli were applied at circadian phases very near the time of the core body temperature minimum. In the consecutive trials for these subjects, the 3 cycle light stimuli were applied at circadian phases during the circadian subjective day. The regression line through the phase response

curve determined from these consecutive trials (slope = -0.61, R = -0.99) was not statistically different from that derived from the steady state trials previously acquired in the laboratory (N = 26, slope = -0.55, R = -0.97).

Regardless of the circadian phase at which the second bright light stimulus was applied, the resulting consecutive trial phase shift was not different from what would be expected from a steady state trial. These data suggest that there is no evidence for transient behavior of light-induced phase shifts in human subjects, and that the human circadian pacemaker is rapidly reset to its final steady state phase following the bright light stimulus used in this protocol. However, it is still conceivable that transient behavior in the phase shifts of the human circadian pacemaker may still be present following other phase shifting stimuli or potentially different light stimulus than used in this protocol (e.g. a single pulse of light). This result contributes to further characterization of the light responsiveness of the human circadian pacemaker and furthers the effective mathematical modeling of the underlying pacemaker mechanism.

Previous studies of astronauts have documented the presence of circadian rhythms abnormalities, sleep disturbances, and vigilance impairment in astronauts even during relatively short flights. A misalignment between the endogenous circadian timing system and the sleep-wake cycle, altogether with erratic exposure to light among astronauts, is thought to be primarily involved in physiologic and behavioral maladaptation to space flight. Therefore, development of countermeasures which result in rapid entrainment of the circadian system to their required work schedule is important and would allow crew members to avoid the performance decrements arising from circadian disruption. Indeed, our preliminary studies suggest that, with careful planning, bright light exposure during the pre-launch period could be done much more efficiently. Refinement of this technology and its incorporation into the work environment of the orbiter could be a significant advance in relieving the deleterious consequences of the extended duty hours and shifting work schedules required during this continuous operation. This will require the induction and maintenance of complete physiologic adaptation of the human circadian timing system to the work schedules required during these missions. The results of the experiments conducted in this project have major implications for understanding the effect of intermittent and/or erratic exposure to light among astronauts during spaceflight. Better understanding of the basic mechanisms underlying this responsiveness to light is necessary to ensure stable entrainment of the circadian system during space flight. These studies will lead to the refinement of Kronauer's mathematical model of photic resetting of the human circadian clock and the design of new lighting regimens to further adjust crew members to their working environment. These analyses will allow us to redirect the task (by modifying the duration and phase of the light stimulus) and to run additional studies. We predict that a properly-timed exposure to a single bright light stimulus or to an intermittent light stimulus prior to lift-off can enable crew members to reduce and/or eliminate the sleep deprivation and consequent fatigue and impaired performance due to misalignment of circadian phase. The present study also has important implications for the treatment of circadian rhythm disorders, since continuous exposure to bright light exposure may not always be achievable in the field. Indeed more than 7 million Americans work at night, either on permanent shifts or on schedules requiring a rotation of day, evening, and night work. These workers forego nocturnal sleep and then attempt to sleep during daytime hours. Yet, complete physiologic adaptation of endogenous circadian rhythms to such inversion of the daily routine usually fails to occur. We conclude that the use of this technology could have a positive effect on the health and productivity of both crew members in space as well as night shift workers here on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Jewett, M.E., Rimmer, D.W., Duffy, J.F., Klerman, E.B., Kronauer, R.E. and Czeisler, C.A. "The human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients." *Am. J. Physiol.* (In Press).

Neural Mechanisms of Adaptation to Altered Gravity

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Funding:

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Post-Doctoral Associates: 1

Responsible NASA Center: Ames Research Center

Task Description:

This work is designed to determine the neural mechanisms underlying sensory-motor adaptation to altered G so that the process can be facilitated or accelerated and "side effects" seen early in the process (e.g., ataxia, motion sickness, disorientation, perceptual illusions, disequilibrium) can be minimized. In the proposed series of studies, the relationship between changes in sensory-motor function (e.g., control of posture and spatial orientation) during adaptation to altered G, and associated changes in morphology, physiology, and neurochemistry of portions of the sensory-motor control systems (e.g., vestibular system, cerebellum, sensory-motor cortex) during adaptation will be determined. Data will be obtained from rats during readaptation to 1-G following chronic exposure to hyper-G produced by centrifugation. The overall goal of these studies is to provide an understanding of the neural processes underlying sensory-motor adaptation to different gravitational environments. Four specific questions will be addressed in this next funding period: (1) Is sensory-motor adaptation to altered G similar to other forms of sensory-motor adaptation (e.g., altered vision, vestibular compensation) in that active, voluntary movement, and previous experience with the altered condition facilitates the adaptation process? (2) What is the significance to the adaptation process of the increased brainstem and cerebellar levels of thyrotropin-releasing hormone (TRH) and Substance P (SP) found following chronic exposure to 2-G? (3) Can sensory-motor adaptation to altered G be facilitated by pharmacological preparations that have been shown to facilitate vestibular compensation? (4) Are the deficits in postural control and spatial orientation seen following adaptation to 2-G the result of a decreased gain in the otolithic portion of the sensory-motor control system? The results of these studies should provide information leading to an understanding of the neural substrate of sensory-motor adaptation to altered G. From this understanding effective behavioral and/or pharmacological methods can be developed to reduce the problems arising from alterations in control of posture, orientation, and movement found during and following long-duration altered G exposure, and to facilitate readaptation to normal G.

The major effort in this funding period has been on electrophysiological studies of the otolith-spinal reflex following hyper-G exposure and following streptomycin treatment. Both of these treatments are known to result in disruption of air-righting and orientation during swimming, behaviors thought to be based on inputs from gravity sensors in the otolith organs. These behavioral findings suggest that sensitivity in the otolithic portion of the vestibular system may be decreased following these treatments. We have used the otolith-spinal reflex response to sudden free-fall to monitor sensitivity in the otolithic system following chronic exposures to hyper-G and streptomycin treatment, hypothesizing that the amplitude of the early EMG response would be lower in treated animals. To monitor the otolith-spinal reflex, the EMG response of gastrocnemius and tibialis anterior muscles of the hindlimb to a sudden, vertical linear acceleration (free-fall) stimulus was used. Analysis of data from the gastrocnemius muscle (data from tibialis anterior muscle is still being analyzed) showed that both hyper-G and streptomycin treatments suppress the otolith-spinal reflex response. This reduction in the otolith-spinal reflex response to sudden free-fall appears to reflect a lowered gain in the gravity sensing portion of the vestibular system. Disruption of air-righting and swimming following treatment with hyper-G or streptomycin may be behavioral manifestations of this reduced gain (Daunton et al., 1997; Taber et al., 1997).

Other studies have been done to modify otolithic inputs to the motor control system, using vestibular galvanic stimulation. One goal of this study is to be able to simulate some influences of hyper-G and micro-G on the neuromuscular system by increasing or decreasing vestibular inputs to the CNS. The first step in this project involved an investigation of areas activated by unilateral galvanic stimulation (Polyakov, et al., 1997).

The results of the proposed studies will have benefits beyond those to NASA. Information derived from these studies will contribute to our understanding of the generic mechanisms that underlie recovery of function following damage to neural systems governing postural and locomotor control. In clinical situations motor control is disrupted by various injuries (e.g., spinal contusion, concussion, cerebral vascular accidents-stroke, vestibular lesions, peripheral nerve damage), as well as disease states (e.g., multiple sclerosis, ALS, cerebral palsy) that affect neuromuscular function. Findings from this integrated approach to studying molecular and functional alterations in the neuromuscular system will lead to improved understanding of the contributions of structures (e.g., motoneurons, cerebellum, vestibular nuclei, motor cortex, proprioceptors) and neurotransmitters (e.g., Substance P, TRH, GABA) to motor control under normal and altered conditions. Results of these studies should contribute to the development of behavioral and/or pharmacological approaches to rehabilitation, thus enhancing the quality of life of individuals affected by injury and/or disease. An understanding of the modifications occurring in the neural substrate during the process of adaptation to altered G will likely provide important insight into the neural mechanisms (e.g., neural plasticity and neuromodulation) involved in adaptation and learning in many non-space situations.

FY97 Publications, Presentations, and Other Accomplishments:

Carcenac, C., Herbuté, S., Polyakov, I., Daunton, N., Malouvier, A., Wronski, T.J., and Gabrion, J. "Choroidal guanylate cyclase activity is altered in rats adapted to either microgravity or hypergravity." (Abstract) *ASGSB Bull.* (In Press).

D'Amelio, F., Fox, R., Wu, L., Daunton, N., and Corcoran, M. "Effects of microgravity on muscle and cerebral cortex: A suggested interaction." *Adv. Space Res.* (In Press).

D'Amelio, F., Wu, L-C., Fox, R.A., Daunton, N., Corcoran, M., and Polyakov, I. "Hypergravity exposure decreases GABA immunoreactivity in axon terminals contacting pyramidal cells in the rat somatosensory cortex." *J. Neurosci. Res.* (In Press).

Daunton, N. "Vestibular galvanic stimulation as a countermeasure for muscle atrophy." Presentation to ARC Board of Directors (May 13, 1997).

Daunton, N., Fox, R., Corcoran, M., Taber, P., Duran, M., and Wu, L. "Suppression of otolith-spinal reflex by chronic hyper-gravity exposure and by chronic streptomycin treatment." (Abstract) *Neurosci. Abstr.*, 23, 754 (1997).

Daunton, N.G., Corcoran, M., Fox, R.A., and Wu, L-C. "Chronic exposure to hyper-G suppresses otolith-spinal reflex in the rat" (Abstract) in "Research and Technology 1996." NASA Ames Research Center (In Press).

Fox, R., Daunton, N., and Corcoran, M. "Study of adaptation to altered gravity through systems analysis of motor control." *Adv. Space Res.* (In Press).

Lowery, M.S., Polyakov, I., and Daunton, N. "Hypergravity effects on muscle fiber type and cross-sectional area in the rat soleus." (Abstract) *Proceedings, Society for Integrative and Comparative Biology* (In Press).

Masseguin, C., Corcoran, M., Carcenac, C., Mani-Ponset, L., Daunton, N., and Gabrion, J. "Aquaporin 1 is down-regulated in rat choroid plexus after altered gravity exposure, as well as after water deprivation and salt-loading." (Abstract) *ASGSB Bull.* (In Press).

Polyakov, I., Kaufman, G., Daunton, N., Fox, R., and Perachio, A. "The effects of galvanic translabyrinthine utricular stimulation on FOS expression in the gerbil brain and spinal cord." (Abstract) *Neurosci. Abstr.*, 23, 1291 (1997).

Wu, L.C., D'Amelio, F., Fox, R., Polyakov, I., and Daunton, N. "Light microscopic image analysis system to quantify immunoreactive terminal area apposed to nerve cells." *J. Neurosci. Meth.*, 74, 89-96 (1996).

Lower Limb Response to Impact Loads in 1G and Micro-G

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Post-Doctoral Associates: 1

Task Description:

Exercise in microgravity is one of the most promising countermeasures to the dual problems of space flight-induced bone loss and muscle atrophy. Although exercise in microgravity has been studied extensively from a metabolic standpoint, little research has focused on the efficacy of different forms of exercise for maintaining musculoskeletal integrity in this unique environment. Exercise protocols thus far have not been effective in preventing muscle atrophy and bone loss during space flight, especially in the lower extremities. In 1-G, however, animal experiments have clearly indicated that (i) certain bone strains and strain rates do stimulate bone deposition, and (ii) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column. Such studies have also indicated that a relatively small number of appropriate loading cycles may lead to bone deposition. This suggests that an optimal exercise regimen might be able to maintain bone and muscle integrity during space flight.

Since there is evidence that the bones and muscles of the lower limbs are particularly affected by space flight, the proposed study will address two major aims: (1) to determine the relationship between (i) externally applied impact loads and rates of loading and (ii) the (global) strains and accelerations in the calcaneus and tibia *in situ* and *in vivo* in 1-G, and (2) to determine the external loads, rates of loading, global strains in the calcaneus, tibial accelerations, and the amount of eccentric and concentric whole-muscle activity during jumping exercises in true and in simulated zero-gravity. Each of these aims will be addressed by well-defined, interrelated experiments. To address the first aim, cadaver experiments will relate the global strains and accelerations in the calcaneus and tibia to each other and to external loads and rates of loading. Subsequent human *in vivo* trials will relate tibial accelerations and global strains in the calcaneus to external loads elicited by jumping exercises. How such loads can be achieved in zero-gravity will be investigated with a simulator that negates the effect of gravity on a subject's limbs. The experiments in the simulator will be validated with KC-135 aircraft experiments in which true zero-gravity is achieved.

The overall goals of this proposal are: (1) to demonstrate that jumping exercises may be more effective and efficient than current exercises performed in zero-gravity with respect to maintaining bone density and muscle

strength; (2) to validate the zero-gravity simulator as an appropriate substitute for true zero-gravity experiments during development of an optimum exercise regime; and (3) to quantify relationships between external loading profiles and internal bone strains. This knowledge will not only benefit planners of an in-flight exercise program, but it is also expected that the novel experimental techniques will provide valuable information in the development of exercise-based countermeasures for osteoporosis and muscle atrophy. Moreover, if the zero-gravity simulator is shown to be an appropriate substitute for true zero-gravity experiments, it will provide a much less expensive way to conduct some experiments.

During FY97 the experimental testing phases for the 1-G impact study and zero-gravity simulator were completed. Cadaver data were collected on fourteen single-leg specimens over a range of peak loads of 300 to 2500N, corresponding to 1 to 5 times body weight. Peak tibial strain ranged up to 0.1% and peak calcaneal strains ranged up to 0.3%. Other investigators who have measured cortical bone strain *in vivo* have reported values of about 0.15% for several animal models and one human experiment. This value also corresponds with the strain level at which microscopic damage development resulting in modulus loss has been observed to begin in tests of human femoral cortical bone. Because of the range of peak loads and tibial strains measured, we have confidence that our test protocol was reasonable and loaded the specimens over a range with an upper limit close to the maximum load that would be encountered in normal circumstances in 1-G. We encountered problems with the original extensometer and resolved them with the design of a new system with lower mass and higher frequency response. The new system gave improved results and was used to capture calcaneal strain *in vivo*. Twelve subjects were tested in the zero-gravity simulator study, four of which were instrumented with the new system. Peak external forces ranged from 1400 to 3100 N, corresponding to 1.7 to 4 times body weight. Test protocol included both two-footed and one-footed landings. All of the subjects could elicit loads under the calcaneus which is important given the fact that there is an absence of heel loading in many exercises currently used during extended orbital missions. On the tests performed to date, we have determined (I) peak loads are comparable to loads produced by jumping exercises *in vivo*; (II) tibial strain data collected from cadaveric limbs are comparable to values reported in the literature (there are no available comparative values for the strains observed in the calcaneus); and (III) analyses indicate statistically significant relationships between external forces and internal bone strains and strain rates. The importance of these findings lies in the fact that we are now in a position to evaluate the osteogenic potential of different exercise countermeasures.

While this research is aimed at developing appropriate countermeasures to prevent bone loss and muscle atrophy in astronauts, it also has applications for humans on Earth. For example, osteoporosis is the degeneration of bone and is a factor in more than 1.5 million fractures per year in the United States. There are many possible mechanisms which cause osteoporosis, one of which is the magnitude of forces applied to the bone. Bone is a living tissue which is constantly breaking down and rebuilding itself. The manner in which it does this is dependent, among many other things, on the loads that it experiences. Activities of daily living, such as walking, are generally associated with forces being applied to the bone which cause the bone to maintain its normal strength. However, when a person is bed-ridden or loses the ability to perform normal activities of daily living, the forces applied to the bones are reduced and thus the strength of the bones is reduced. This results in a danger of bones breaking under circumstances in which they would not normally break, such as tripping or falling.

Zero gravity induced osteoporosis can be examined to investigate the amount of force necessary to maintain normal bone mass. Thus, efforts to determine the stimuli (in terms of magnitude of force, the rate of force application, and the frequency of the force application) necessary to prevent bone loss in astronauts will also provide us with a basic understanding of the factors affecting bone formation/degradation in general and will aid in our understanding of osteoporosis and measures which may be undertaken to prevent or slow down this process. Likewise, an understanding of muscle atrophy in astronauts and appropriate measures to counteract this loss will translate to a greater understanding of muscle atrophy in the general population, such as that associated with disuse or aging.

FY97 Publications, Presentations, and Other Accomplishments:

Cavanagh, P.R., Davis, B.L., Grabiner, M.D., and Gross, T.S. "Effects of microgravity on the musculoskeletal system." *Med. Sci. Sports Exercise*, Vol 29, 5, Abstract #1367, S241 (1997).

Courtney, A.C., Perusek, G.P., Davis, B.L., Sferra, J., and Kambic, H.E. "External impact loading and tibial strains in cadaveric lower limbs." XVI meeting of the International Society of Biomechanics, Tokyo, Japan (1997).

D'Andrea, S., Davis, B.L., Courtney, A.C., and Perusek, G.P. "External impact loads on the lower extremity during jumping in simulated microgravity and the relationship to internal bone strain." The 12th Man in Space Symposium: The Future of Humans in Space, Washington, DC (June 8 - 13, 1997).

D'Andrea, S., Lord, D., and Davis, B.L. "A rheological model of the human heel pad." The American Society of Biomechanics meeting, Clemson, SC (September 24 - 27, 1997).

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Acoustic Bone Mass and Trabecular Property Measurements

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Funding:

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Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$58,493

Task Description:

The proposed ground-based research is for development of non-invasive, nonhazardous, subsonic, and ultrasonic techniques for bone property measurements. The innovation of the proposed study is the use of nonlinear acoustic testing techniques and a combination of ultrasonic and infrasonic techniques to measure and monitor micro and macro changes in bone conditions. The proposed project will lead to the development of light-weight, compact, and relatively inexpensive instruments which can be used during space flight as well as for ground-based research.

FY97 efforts were focused on ultrasonic measurements of the nonlinear parameter of the trabecular bones. A number of ultrasonic tests were carried out and the frequency range from 1 kHz to 800 kHz was studied. Different experimental setups were constructed to accommodate this wide frequency range. One of the major difficulties in performing the nonlinear measurements is the nonlinear interference in electronic equipment. To avoid such interference, each setup was carefully designed and calibrated. In addition, the nonlinear parameter independently measured with different setups were compared and only agreed measurements were taken into account. Various approaches to measure the nonlinear parameter were tested. It appeared that the most reliable approach is to measure interaction of higher frequency ultrasound with lower frequency vibration. One series of the tests was performed at a water tank. Bone samples (half inch thick slices of a bovine trabecular bone) were submerged into water in order to provide good coupling with the ultrasonic signals. Amplitude modulated signals were employed in this test. After transmitting the AM signal through the sample, a part of the signal energy transforms into the low frequency (the frequency of modulation) signal due to the nonlinearity of the sample. The more the nonlinear parameter of the sample, the more intensive such a transformation. This low frequency signal is then picked up with a hydrophone (receiver), filtered, digitized, and measured.

The other tests were performed in air on larger pieces of bone (over 5 inches long) attaching sensors directly to the bone. The effect of modulation of higher frequency ultrasound with lower frequency vibration was utilized to measure the nonlinear parameter. The same high frequencies and low frequencies were used in both setups, so the independently measured nonlinear parameters of the samples (cut from the same bone) could be compared. The two experimental approaches and setups produced the most reliable measurements due to the simplicity of the electronic circuitry minimizing nonlinear interference in the circuitry.

High dissipation of ultrasonic waves in trabecular bone in the frequency range above 100 kHz made it difficult to measure the nonlinear effects. We were able to reliably measure the nonlinear parameter in the frequency range below 100 kHz. Thus, the nonlinear parameter was measured for three ultrasonic frequencies 26, 37, and 60 kHz modulated by the vibration frequencies 4.6 and 9.8 kHz. *In vitro* determined values of the nonlinear parameter for bovine bone used in these tests was in the range 80 - 120, which is an order of magnitude higher than for non-porous media (e.g., compact bone and liquid). At this point we are confident in reliability of the experimental approach and measured data, and are working on increasing the precision of the technique and establishing the correlation between the measured nonlinear parameter and the structural parameters of trabecular bones.

In addition to the experimental studies, we also made advancements in theoretical modeling of the liquid-saturated porous media such as trabecular. The derived theoretical approach is based on Biot's semilinear model of porous media. This model assumes that liquid and solid matter of the porous medium exhibit linear behavior within a wide practical range of stresses. On the other hand, the strain due to the dynamic (acoustic) stresses involves modification of local geometry in the pores. These modifications are essentially nonlinear even for small strains. Such mixed behavior leads to nonlinear stress-strain relations. The important result of the derived nonlinear model is that it establishes a correlation between the measurable effective nonlinear parameter and the structural parameters of the medium. Specifically, the strong correlation between the effective nonlinear parameter and porosity of the medium is shown.

The measured high level of the nonlinear parameter of the trabecular combined with the theoretical finding on correlation between the nonlinear parameter and structural parameters of the bone proved the concept of the proposed approach that the measurements of the nonlinear parameter can serve for assessment of the trabecular properties.

The project should lead to development of innovative techniques and instruments to assess human bone quality and may allow for diagnosis of osteoporosis. The techniques can be used by general practitioners, physicians, and rehabilitation specialists.

The developed experimental technique and theoretical model, in addition to trabecular bone assessment, can also be applied for other porous media such as sea sediments, stones, and soil. This can be used for seismo-prospecting and geophysical research.

FY97 Publications, Presentations, and Other Accomplishments:

Donskoy, D.M. "Infrasound method for bone mass measurements." *J. Acoust. Soc. Am.*, (5), 101 (1997).

Patent Approved, U.S. Patent Application #: 6,000,474. Donskoy, D.M. "Non-invasive technique for bone mass measurement."

Donskoy, D.M. and Sutin, A.M. "Nonlinear acoustic parameter of trabecular bone." *J. Acoust. Soc. Am.*, 102 (1997).

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Modulation of Bone Remodeling via Mechanosensitive Channels

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Funding:

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Students Funded Under Research: 1
FY 1997 Funding: \$118,884

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 0

Task Description:

We have characterized a mechanosensitive channel (SA-cat) in osteoblasts which we propose is involved in the signaling mechanism for converting physical strain into osteogenic responses. We previously found that high magnitudes of chronic, intermittent mechanical strain (CMS) alters SA-cat channel kinetics. In the original grant, we proposed to examine the effects of different magnitudes and frequencies of CMS on SA-cat channel kinetics, intracellular calcium, interaction with calciotropic hormones and integrin-cytoskeletal interaction. However, recently we have developed a mechanical loading apparatus which can alter the levels of strain and fluid shear independently. Using this apparatus, we have found that fluid shear, and not physiologic levels of mechanical strain, affected the osteogenic marker, osteopontin, as well as c-fos and COX-2 expression and prostaglandin synthesis. Therefore, we have altered this grant to include examination of the effects of fluid forces on osteoblast function. In these studies we plan to apply different levels of fluid shear and mechanical strain to osteoblasts and osteoblast-like cells *in vitro* and in conjunction with patch clamp analyses and cellular and molecular techniques, examine the role of mechanosensitive channels in: 1) the osteoblastic response to varied magnitudes and frequencies of both strain and fluid shear, and the interaction of these two types of mechanical stimuli; 2) the intracellular calcium response to these mechanical stimuli; 3) the interaction of fluid shear and strain with hormonal stimulation on osteoblastic function; and 4) the relationship of these mechanical stimuli with the extracellular matrix-integrin-cytoskeletal axis. These studies will provide important information on how bone responds to the mechanical environment and perhaps, how the loss of mechanical stimulation, as in weightless conditions, alters calcium balance during extended space flights.

During this funding period, we made significant progress on each of the specific aims of the grant. As stated in the last fiscal year report, we have found that the effects of fluid shear on the bone anabolic marker, osteopontin, are much greater than those of physiological strain. Using a new device based on the four point bending *in vivo* model of mechanical loading, we were able to apply fluid force or physiologic strain independently. We found no effect on osteopontin message resulting from strains below 5000 microstrain, however fluid forces increased osteopontin message 4-5 fold over non-loaded controls. Subsequent studies examining possible signaling pathways have shown similar effects of fluid forces on c-fos, cyclooxygenase-2 and TGF β expression following 0.5, 3, and 6 hours of loading, respectively.

We have tentatively identified the mechanosensitive channel found in UMR-106.01 cells. Using an antisense oligodeoxynucleotide strategy, we found that transfection of UMR cells with antisense against the α_1 subunit of the cardiac isoform of the L-type, dihydropyridine-sensitive Ca^{2+} channel completely blocked the mechanically-induced whole cell conductance. During this funding period, we have used the four point loading system to examine the effects of fluid forces and physiologic strain on molecular and functional expression of these channels in osteoblast-like cells. In collaboration with Dr. Mary C. Farach-Carson at the University of Texas-Houston, Dental Branch, we used rt-PCR techniques with primers directed against the α_1 subunits of the cardiac isoforms of the L-type Ca^{2+} channel to find that a mechanosensitive isoform is increased 5 fold following 24hr of mechanical loading in MC3T3-E1 cells. We have also determined via patch clamp techniques that whole cell currents are increased 7 fold. By independently varying fluid forces and strain, we determined that, like the increase with osteopontin and other signaling factors, the upregulation of this mechanosensitive channel is mediated by fluid forces and not physiologic levels of strain. Furthermore, using a recently purchased Ca^{2+} imaging system, we have demonstrated that this increase in channel expression correlates to an increase in intracellular calcium.

In collaboration with Dr. Fred Pavalko at the Indiana University Medical Center, we have also examined the effects of fluid shear on the cytoskeleton and integrins in osteoblastic cells. Using a continuous laminar flow apparatus, we found that fluid shear dramatically rearranges the actin cytoskeleton. Within 40 min of application of shear, extensive stress fiber formation is observed, correlating with intense staining for the integrin subunit, β_1 , and the actin associated protein, α actinin. Furthermore, we demonstrated that continuous fluid shear also increases c-fos and COX-2 mRNA expression in a manner similar to that we observed with the four point bending system. α actinin has been shown to bind the integrin subunit to the actin cytoskeleton. Dr. Pavalko has developed a fragment of α actinin which binds to the integrin subunit but does not have the region to bind to the actin filaments. Microinjection of this fragment prevents the formation of actin stress fibers in response to shear. Significantly, the increase in c-fos and COX-2 expression in response to shear was abolished with the prevention of stress fiber formation. These data would suggest a direct link between changes in cytoskeletal organization and gene expression.

We also collaborated with Dr. Farach-Carson to examine the interaction of the mechanosensitive channel with the typical L-type Ca^{2+} channel in response to stimulation with calciotropic hormones. Previously, Dr. Farach-Carson demonstrated that $1,25(\text{OH})_2$ vitamin D_3 altered L-type Ca^{2+} channel kinetics by decreasing the amount of depolarization required to activate the channel and increase open time of the channel. We have previously demonstrated that PTH alters the kinetics of the mechanosensitive, or SA-cat, channel in osteoblasts, leading to a depolarization of the membrane. In this study, we used Ca^{2+} imaging to determine the effects of the two hormones on intracellular Ca^{2+} signaling. We found that $1,25(\text{OH})_2$ vitamin D_3 alone was unable to induce an increase in $[\text{Ca}^{2+}]_i$ in the osteoblast-like cell line, MC3T3-E1. PTH produced a slight increase in $[\text{Ca}^{2+}]_i$ in these cells. However, when MC3T3-E1 cells were pretreated with $1,25(\text{OH})_2$ vitamin D_3 2-10 min prior to addition of PTH, the increase in $[\text{Ca}^{2+}]_i$ was significantly elevated. Inhibitor studies demonstrated that block of the L-type Ca^{2+} channel produced a $[\text{Ca}^{2+}]_i$ response similar to PTH alone when both hormones were added. Addition of the SA-cat channel blocker, Gd^{3+} , completely abolished the $[\text{Ca}^{2+}]_i$ response to these hormones. These data indicated that $1,25(\text{OH})_2$ vitamin D_3 altered the L-type Ca^{2+} channel so that the depolarization induced by PTH via activation of the mechanosensitive channel could significantly increase L-type Ca^{2+} channel activation. These data also suggest that these two hormones act in concert through two types of channels to produce a highly significant signal. These data further suggest that calciotropic hormones may modulate the sensitivity of bone to mechanical stimulus, resetting the threshold of activation so that lower magnitudes of loading could produce a greater response.

The mechanical environment is vital to the function of many physiologic systems, but perhaps none as key as to bone. Removal of mechanical stimulus, as in immobilization or space flight, produces a rapid loss of total body calcium, a decrease in bone matrix proteins, and a reduction in bone mass, ultimately producing an osteoporotic condition termed immobilization osteoporosis. Conversely, application of mechanical stimulus to

bone increases bone mass and can retard bone loss induced by other pathologies, such as postmenopausal osteoporosis. Illumination of the cellular mechanisms responsible for the transduction of mechanical stimuli into cellular biochemical responses will provide both physiologic and pharmacologic foci to attempt to provide methods to increase bone formation, a critical medical concern to the aging population. In addition, recent evidence has demonstrated that interaction of mechanical stimulation with calcitropic hormones suggest that this interaction could alter the thresholds of mechanical stimulation to increase bone mass. With the possibility of conservation of mechanotransduction mechanisms in other systems, these studies could provide valuable insight into medical problems such as hypertension.

FY97 Publications, Presentations, and Other Accomplishments:

Duncan, R.L. "Mechanosensitive ion channels in osteoblasts: Identification of a possible mechanotransduction pathway" in "Frontiers of Biological Science in Space: Molecular Mechanisms of the Gravity Response in Cells." Edited by: Sato, A. and Noda, M. Tokyo University Press, Tokyo, Japan (In Press).

Duncan, R.L., Akanbi, K.A., and Farach-Carson, M.C. "Calcium signals and calcium channels in osteoblastic cells" in "Seminars in Nephrology." Edited by: Kurtzman, N.A. W.B. Saunders Co., Philadelphia, PA, IVth Update in Renal Physiology (In Press).

Duncan, R.L., Ryder, K.D., Akanbi, K., and Farach-Carson, M.C. "Comparison of fluid forces and strain on mechanosensitive currents in osteoblast-like cells." (Abstract) FASEB J., 11(Suppl.), A329 (1997).

Duncan, R.L., Ryder, K.D., Akanbi, K., and Farach-Carson, M.C. "Effects of fluid shear and mechanical strain on ion channels in osteoblasts." (Abstract) Ann. Biomed. Eng., 25(Suppl 1), S-76 (1997).

Li, W., Duncan, R.L., Karin, N.J., and Farach-Carson, M.C. "1,25 (OH)₂D₃ enhances PTH-induced Ca²⁺ transients in pre-osteoblasts by activating L-type Ca²⁺ channels." Am. J. Physiol. (Endocrine), 273, E599-E605 (1997).

Owan, I., Burr, D.B., Turner, C.H., Qiu, J., and Duncan, R.L. "Osteoblasts do not respond to physiological levels of mechanical strain, but are responsive to fluid effects." (Abstract) Transactions of the 43rd Annual Meeting of the Orthopaedic Research Society, Vol. 22: 176 (1997).

Owan, I., Burr, D.B., Turner, C.H., Qiu, J., Tu, Y., Onyia, J.E., and Duncan, R.L. "Mechanotransduction in bone: Osteoblasts are more responsive to fluid forces than mechanical strain." Am. J. Physiol. (Cell), 273, C810-C815 (1997).

Turner, C.H., Owan, I., Tu, Y., Onyia, J.E., and Duncan, R.L. "Regulation of osteoblast gene expression by mechanical strains and fluid forces." (Abstract) FASEB J., 11(Suppl.), A287 (1997).

Turner, C.H., Owan, I., Tu, Y., Onyia, J.E., and Duncan, R.L. "Regulation of osteopontin and c-fos genes by mechanical forces." (Abstract) J. Dent. Res., 76(Suppl.), 377 (1997).

Postural Effects on PTH, Calcium, and Skeletal Dynamics

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Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Marked bone demineralization and severe hypercalciuria resulting in an increased risk for stone precipitation are two salient features of the weightless state. The mechanisms responsible for these changes are unclear.

Project 1: PTH and bone resorption circadian rhythms.

Previous studies unraveled a number of abnormalities in response to the weightless state including disturbances in the amplitude and period of various circadian rhythms. There is an increasing body of evidence supporting an anabolic effect of parathyroid hormone (PTH) circadian rhythm on bone remodeling, either directly through its effects on bone remodeling or indirectly through decreasing urinary calcium excretion. The objective of the project is to demonstrate that PTH rhythm is truly endogenous, is affected by posture, and that both parameters have a significant impact on bone resorption.

Project 2: Effect of postural changes on urinary calcium and sodium handling.

The essential role of the kidney in calcium balance is undisputed and significant alterations in renal hemodynamics and sodium homeostasis have been noted during simulation of the weightless state. The objective of this study is to demonstrate that serum ionized calcium levels tightly modulate calcium and sodium excretion, possibly through the calcium sensor. These observations propose a novel mechanism that may partially explain the bone demineralization and the decrease in extracellular volume that takes place with the weightless state.

Project 1: We recently implemented a constant routine protocol (CR), and demonstrated that when subjects are semirecumbent, fed hourly meals, and kept in a state of forced wakefulness, PTH diurnal rhythm is truly endogenous (El-Hajj Fuleihan et al, *JCEM* 1997). However, under the above conditions, its amplitude is blunted and the amplitude of urinary calcium excretion is increased. Bone resorption, as assessed through N-telopeptide cross-links (N-Tx), also followed an endogenous diurnal rhythm, with a mean level during the CR that was significantly higher than that during the baseline day (74.1 and 59.1 nM BCE/mMCR respectively, $p =$

0.003 by t-test). During the CR, there was a significant positive correlation between N-Tx and PTH, $R = 0.928$ at a lag of $\bar{n}5.75$ hr (PTH precedes N-Tx).

The impact of changes in posture per se on the above mentioned rhythms is, however, unclear. In this project we sampled PTH, ionized calcium (Ca_i), and other serum and urinary indices of mineral and bone metabolism every 20-60 min for 24-36 hours under baseline conditions followed by 40 hours of constant posture (CP) in 10 healthy male subjects. Under CP, there was a blunting in the amplitude of PTH circadian rhythm that was accompanied by a marked increase in urinary calcium, sodium). The fact that mean serum Ca_i levels throughout the CR decreased during the CP suggest that the primary event is not increased bone resorption but a blunting in PTH amplitude, decreasing its anabolic effect, thus resulting in increased bone resorption and enhanced urinary calcium excretion. The above mentioned changes in the indices of calcium and bone metabolism took place very early on during the onset of CP, a protocol partially simulating weightlessness, suggesting that the blunting in PTH amplitude and the catabolic bone remodeling profile occurs very early during the loss of gravitational forces.

Project 2: Most studies evaluating urinary calcium handling by administering calcium have been limited by the fact that as Ca_i levels increase, PTH levels gradually decrease; studies were also limited by the fact that indices of renal hemodynamics (that could affect calcium handling) were not evaluated. We have developed a calcium handling protocol, monitoring renal blood flow and glomerular filtration rate, under a PTH clamp to evaluate Ca_i -dependent (PTH independent) renal calcium handling. Its implementation, in eight subjects studied during the semirecumbent posture, revealed that not only urinary calcium but also urinary magnesium and sodium excretion are all tightly modulated by increments in Ca_i levels (El-Hajj Fuleihan, *JCEM* in press), possibly through the calcium receptor. These observations suggest that the calciuresis and natriuresis documented in the weightless state may be closely modulated by changes in serum ionized calcium levels and urinary calcium excretion outlined in our first project. We therefore propose a novel mechanism that may partially explain the skeletal demineralization and decrease in extracellular volume that takes place with the weightless state.

Our protocols shed important light on the mechanism of immobilization hypercalcemia, hypercalciuria, and bone loss. We demonstrated that the blunting of the amplitude of PTH rhythm in response to the semirecumbent posture is accompanied by hypercalciuria and a catabolic bone remodeling profile. Measures aiming at increasing PTH amplitude through PTH administration and/or use of Fosamax will further elucidate the specific role of PTH circadian rhythm on calcium and bone metabolism and will confirm whether these therapeutic strategies reverse the deleterious metabolic changes that occur in the weightless state, herein simulated by changes in posture.

Similarly, we have developed a protocol that specifically characterizes calcium-dependent calcium and sodium excretion. There was increased renal calcium and sodium excretion in the semirecumbent posture. Our findings suggest that this effect is mediated through the calcium receptor. These studies need to be implemented in the erect state to determine the impact of posture on these changes. The use of medications targeted at the calcium receptor will reverse the negative calcium balance state that may ensue from the weightless state.

The above mentioned therapies are applicable to immobilization hypercalcemia and idiopathic hypercalciuria. Once our models are validated to represent biological changes which take place in space, these therapies could also be used to prevent the bone loss experienced by astronauts in space. Finally, such therapies may also have a significant impact on the development of treatment strategies for osteoporosis, a disease affecting 1/3 of women and a significant number of men by age 90. Osteoporosis results in a staggering cost to the health care system of the United States of America. It is estimated to incur an expenditure of 10 billion dollars annually, a number that is on the rise due to the increasing elderly population.

In summary, our studies will bring a new dimension to our understanding of some of the mechanisms responsible for both space flight-induced as well as idiopathic osteoporosis and nephrolithiasis, thus allowing the development of novel countermeasure programs to prevent these processes.

FY97 Publications, Presentations, and Other Accomplishments:

El-Hajj Fuleihan, G., Klerman, E.B., and Brown E.N. "The parathyroid rhythm is truly endogenous." *J. Clin. Endocrinol. Metab.*, 82, 281-286 (1997).

El-Hajj Fuleihan, G., Klerman, E.B., Brown, E.N., and Czeisler, C.A. "N-Tx diurnal rhythm is truly endogenous." *J. Bone Min. Res.*, 12(Suppl 1), S613 (1997).

Cardiopulmonary Hemodynamics in Microgravity

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Funding:

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Task Description:

Ground-based studies have shown a relationship between central venous pressure and cardiac output. In addition, simulations of microgravity have shown that cardiac output increases initially and then returns to control levels over a two to three hour period, over which time blood pressures are tightly regulated at about one Gz levels. The reduction in cardiac output in simulated gravity has been assumed to be due to a reduction in plasma volume; however, this has not been universally demonstrated. Cardiac output and pulmonary blood flow are equal and the distribution of blood flow in the lung is believed to be gravity-dependent. Based on this discussion, during microgravity, pulmonary blood flow should increase with an increase in capillary recruitment, and thus blood flows to the upper parts of the lung. Under this condition, the lung's distribution would be more homogeneous. Results from recent space flight experiments demonstrated that cardiac output was increased in space. In spite of an absence of an increase in central venous pressure, cardiac output remained elevated for 14 days and cardiogenic oscillations were evident, suggesting that lung blood volume was heterogeneous. Based on these observations, the present study was designed to determine cardiac function and blood volume distribution in the lung using nuclear medicine techniques. The specific hypotheses were that removing gravity would result in: 1) an increase in end-diastolic volume, although the effect on cardiac output would be blunted by a decrease in sympathetic tone and contractility resulting in an increase in end-systolic volume and compliance. If true, this could explain how cardiac output could be elevated in space without an increase in central venous pressure and why cardiac output remained elevated in space (increased sympathetic tone); and 2) the increase in pulmonary blood flow and volume would be accommodated by a decrease in pulmonary resistance caused by vasodilatation of lung blood vessels. This would imply that the nature of blood volume distribution in the lung would not change with gravity, the heterogeneity of blood volume would remain, and the reduced pulmonary vascular resistance could play a role in the central venous pressure.

In the two previous years of this project, we have demonstrated that cardiac output is increased during periods of simulated reduced gravity and decreased during periods of increased simulated gravity. In fact, the changes in

cardiac output followed linearly the changes in gravity; however, the changes in cardiac output were less than would be expected from the gravitational changes. The blunted response to gravity was due to compensation in cardiac contractility mediated by sympathetic tone. In addition, the change in contractility was parallel by a change in cardiac relaxation time measured from cardiac filling (compliance). These changes in cardiac function can explain, in part, the absence of association between cardiac output and central venous pressure observed in space on previous flights. Although this modulation regulates blood pressure, it reduced cardiac work in space and increased cardiac work in hypergravity. Although periods of increased gravity are short, long periods of hypogravity may lead to cardiac deconditioning due to the reduced sympathetic tone. These studies also showed that the vasodilatation in hypogravity and vasoconstriction in hypergravity affect the pulmonary circulation as well. The blood volume distribution in the lung is heterogeneous at one Gz, and remained so during experiments where the Gz was increased (lower body negative pressure) and decreased (lower body positive pressure). These data imply that there was a reduction in pulmonary vascular resistance. The change in pulmonary vascular resistance needed to explain these data are small as the pulmonary arterial pressure is low. The changes in blood volume distribution and pulmonary vascular resistance can explain the lower central venous pressure observed in space and suggest that during hypergravity the central venous pressure may be held higher than expected.

The experiments conducted in the first two years led to the experiments conducted in the last year, where cardiac and pulmonary parameters were measured in hypergravity conditions. Twelve subjects were studied under conditions of increased gravity in a human centrifuge. Seven of these subjects completed experiments at 0, 1, 2, and 3 Gz. Measurements of cardiac output, heart rate, and blood pressure were determined at each level of Gz. Due to the technical limitations imposed by the centrifuge set up pulmonary measurements were limited to ventilation, oxygen consumption, and diffusing capacity. These data were analyzed using Analysis of Variance for Repeated Measures.

Cardiac output increased from 6.6 l/min to 8.9 l/min going from standing to the head down tilt position. Increasing gravity from 1 to 3 Gz resulted in a linear decrease in cardiac output to a value of 3.2 l/min at 3 Gz. The decrease in cardiac output was parallel to progressive changes in stroke volume from 141.3 ml at 0 Gz to 32.5 ml at 3 Gz. Heart rate increased from 65 b/min at 0 Gz to 125 b/min at 3 Gz.

Mean arterial pressure increased exponentially from 95 mmHg at 0 Gz to 120 mmHg at 3 Gz. This increase was a result of both an increased systolic (125 to 145 mmHg) and diastolic (70 to 110 mmHg) blood pressure. Pulse pressure decreased linearly from 56 mmHg at 0 Gz to 37 mmHg at 3 Gz. The increase in diastolic and mean arterial pressure was due to an increase in total peripheral resistance, which increased .0100 exponentially to .0300 at 3 Gz.

In spite of the decrease in pulmonary blood flow at increased Gz, functional residual capacity remained unchanged (did not increase as predicted). In contrast, diffusing capacity decreased as pulmonary blood flow decreased; however, when pulmonary blood flow was increased (head-down-tilt) diffusing capacity did not increase as predicted. Both capillary recruitment and engorgement decreased with increasing Gz, accounting equally for the reduction in diffusing capacity.

The data from the centrifuge experiments are qualitatively different from the data of our previous experiments using simulated increased gravity (lower body negative pressure). Specifically we did not observe the expected increases in functional residual volume at increased Gz that we expected with the reduction in pulmonary blood flow that was observed. This may be due to changes in chest wall mechanics observed at Gz and/or changes in the distribution of ventilation and blood volume. We were unable to measure pulmonary blood volume in these experiments, however diffusing capacity decreased with decreasing pulmonary blood flow as expected. The changes in cardiac variables during increased Gz were as expected during increased Gz.

In summary, changes in simulated gravity and actual gravity produce different results on the cardiopulmonary system. The initial changes in blood pressure are modulated through the baroreceptor system to dampen the increased pressure at 0 Gz and increase pressure at increased Gz to maintain resting perfusion levels. Under these

conditions, the distribution of the perfusion in the lung would appear to be altered. It would appear that in situations where there is increased pulmonary blood flow, the distribution of blood volume remains heterogeneous. This would occur as the pulmonary vascular resistance is decreased by dilating arterioles (thus capillaries) that are already recruited, as opposed to recruiting more capillaries. During increased Gz it would appear that the heterogeneity of blood volume distribution is exaggerated due to vasoconstriction and reduced perfusion pressure. These data have implications for pulmonary function during long-term space flight as the extension of pulmonary capillaries and cardiac output would appear to persist through out the duration of space flights. The impact of these two parameters needs further analysis.

Our present physiological and medical understanding of the heart is based on a relationship between central venous pressure and cardiac output and that the distribution of blood in the lungs is thought to be gravity-dependent. The recent data from space flight experiments suggests that these two premises may not be true, and the results of these experiments add support to validity of our previous space flight data. The changes in how cardiac function and pulmonary perfusion are viewed could have an effect on physiology and medical diagnosis and treatments. A direct application of the data is to people with heart failure, venous insufficiency, and lung diseases. These pulmonary blood volume experiments may help us understand the pulmonary edema that is associated with some medical diseases like heart failure and pulmonary diseases and syndromes like mountain sickness.

Magnetic Resonance Imaging in Assessing Forearm Muscle Fatigue after EVA-Related Tasks

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Funding:

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Students Funded Under Research: 0
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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Responsible NASA Center: Johnson Space Center

Task Description:

Forearm fatigue is one of the limiting factors of longevity during extravehicular activity (EVA). Quantification of forearm muscle fatigue is necessary to guide design of extravehicular mobility unit (EMU) gloves, EVA tools, and exercise protocols for training EVA crew members. Magnetic resonance imaging (MRI), when enhanced by previous exercise of the muscle under study, can differentiate muscle groups from each other and from adjacent fat and connective tissue. Using MRI, specific muscle groups most used during EVA-related hand tasks, and therefore most essential for the performance of EVA, can be identified. Also, magnetic resonance spectroscopy (MRS) can be used as a non-invasive means of determining adenosine diphosphate concentrations in the forearm musculature. We hypothesize that MRI T2 and MRS signal intensities can be used as objective, reliable measures of forearm muscle use.

Evaluation of 6 subjects was completed in which a specific EVA type task protocol showed that the use of T2-weighted MRI of the forearm musculature is a valid method of assessing the role of individual muscles in performance of the specific task. Both T2-weighted and high resolution magnetic resonance images were obtained and compared in order to relate the areas of high muscular activity in performance of the task with anatomic localization. Future work will include phosphorous spectroscopy (MRS) in order to more fully elucidate the specific level of metabolism of individual muscles in performance of specific EVA-related hand tasks.

The results of this investigation will provide new information which may be important in a number of situations including physical assessments of occupational tasks of a repetitive nature which are known to result in muscle fatigue and injury (i.e., keyboard input, assembly line work, sports activities). Physical therapy and

occupational rehabilitation of these types of repetitive use injuries could be mitigated by knowledge and techniques validated in this study. Additionally, information gained by study of forearm muscle use in repetitive activities could be used in the design of human-machine interfaces in order to attenuate muscle fatigue and injury potential.

Limb Muscle Function with Unloading and Countermeasures

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-26-17-08

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 6

Post-Doctoral Associates: 1

FY 1997 Funding: \$143,378

Task Description:

Our primary objectives are to: (1) characterize the cellular effects of the hindlimb suspension (HS) model of weightlessness on the functional capacity of single limb skeletal muscle fibers; (2) continue studies designed to elucidate the mechanism of how HS alters substrate metabolism and increases fatigue; and (3) determine the effectiveness of various countermeasures in the prevention of muscle cell atrophy and the associated functional changes such as the loss of force and power.

The overall goal of our research is to understand how weightlessness and models of weightlessness alter the functional capacity of limb skeletal muscles, and develop effective exercise countermeasures to prevent muscle atrophy and the known deleterious changes associated with the atrophy process. In this work, we are employing the hindlimb suspension (HS) rat model to study the cellular properties of individual fibers isolated from the soleus muscle. Our primary efforts during FY97 have been: (1) cellular studies to determine the mechanism of the HS-induced increase in the maximal shortening velocity (V_o); (2) countermeasure studies evaluating the effectiveness of heavy isotonic weight lifting; and (3) studies designed to determine the mechanism for the increased dependence on cell glycogen and the reduced free fatty acid oxidation during exercise following HS. Regarding task 2, we had previously observed that heavy isotonic weight lifting actual resulted in a reduced peak force for the slow type I fibers of the soleus compared to fibers from animals that under went HS only. The weight lifted was 180 to 210 % of body weight, and we hypothesized that this heavy load may have induced fiber damage during the eccentric phase of the lift. Consequently, we repeated the study with somewhat lower loads (130% to 180% of body weight). The exercise consisted of a voluntary, squat-type resistance exercise, and the rats were trained twice daily for two weeks prior to HS. Just prior to HS, rats would perform an average of 42 repetitions per day, lifting ~175% of body mass. During the 2 wk HS period, the number of reps declined to 26 per day and the amount of weight lifted declined to ~130% of body mass. The exercise prevented 70% of the HS-induced loss of soleus muscle mass and completely restored the soleus mass to body mass ratio. Peak force of the slow type I fiber following HS, HS plus standing, and HS plus weight lifting was decreased by 42%, 36%, and 22% respectively compared to control fibers. All of the decline in force could be accounted for by the decrease in fiber size, as the force per cross-sectional area was not different between groups. These results indicate that isotonic weight lifting can attenuate but not prevent the HS-induced decline in peak force and power. The possibility exists that isometric exercise or isometric exercise in combination with isotonic exercise

might be more affective. Currently, we are beginning to test these possibilities and document the muscle recruitment patterns obtained with each exercise with EMG recordings. Regarding task 3, we studied the effects of 14 days of HS on the ability of the soleus and gastrocnemius to oxidize pyruvate and palmitate. Similar to the space flight, HS had no effect on pyruvate oxidation. However, different from the space flight results, we observed only small and non-significant declines in the ability of either the soleus or the gastrocnemius to oxidize palmitate. These results suggest that at least for HS the increased dependence on carbohydrate metabolism during exercise cannot be due to a depressed content of any of the enzymes involved in fatty acid metabolism. Thus the most likely explanation for the HS-induced increase in glycogen metabolism is the combined effects of substrate level activation of glycolysis and inhibition of fat oxidation - this possibility is currently being studied.

A major goal of this research is to elucidate the functional changes associated with zero G-induced muscle wasting and develop exercise countermeasures. The program is essential to our ability to explore the universe and work successfully in space. Stated another way, we simply can not embark on long-term space travel until we can understand and prevent muscle wasting. Similar types of muscle atrophy occur on Earth in various muscle diseases and during the normal aging process. This work will provide an increased understanding of basic muscle function, and how it is deleteriously altered with inactivity. Furthermore, it will result in the development of new exercise protocols and strategies that should be more effective than current procedures in slowing atrophy associated with the aging process. Since one of the main problems encountered by older adults is weakness which leads to debilitating falls, these modalities will improve the quality of life and will lead to considerable savings in medical costs.

FY97 Publications, Presentations, and Other Accomplishments:

Bangart, J.J., Widrick, J.J., and Fitts, R.H. "Effect of intermittent weight bearing on soleus fiber force-velocity-power and force-pCa relationships." *J. Appl. Physiol.*, 82, 1905-1910 (1997).

Kidd, K., Grichko, V.P., and Fitts R.H. "Substrate oxidation rates in rat heart and fast and slow skeletal muscle: Effect of hindlimb unloading." *Med. Sci. Sports Exercise*, 29, S183 (1997).

Widrick, J.J. and Fitts, R.H. "Peak force and maximal shortening velocity of soleus fibers after non-weight-bearing and resistance exercise." *J. Appl. Physiol.*, 82, 189-195 (1997).

Widrick, J.J., Romatowski, J.G., Karhanek, M., and Fitts, R.H. "Contractile properties of rat, rhesus monkey, and human type I muscle fibers." *Am. J. Physiol.*, 272 (Regulatory Integrative Comp. Physiol. 41), R34-R42 (1997).

Effect of Bed Rest on Simulated Shuttle Emergency Egress

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Funding:

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Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1997

Students Funded Under Research: 1

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Responsible NASA Center: Johnson Space Center

Task Description:

Astronauts have never been required to perform an actual emergency egress from the space shuttle. A MODE 5 (unassisted) egress would occur after a landing where the usual ground support crew cannot assist the astronauts in egressing the vehicle. In the ground-based part of this study, human test subjects will be trained in performing the egress procedures. In preliminary data, even without space flight, many persons cannot complete the emergency egress scenario with actual flight hardware. The purpose of this study is to evaluate responses during egress simulation to ascertain whether failure to complete the egress scenario is due to: 1) build-up of CO₂ in the non-conformal helmet; 2) leg fatigue and ischemia associated with wearing an inflated g-suit while walking; or 3) overheating. In the flight portion of this study, crew members will be asked to walk on a treadmill immediately after shuttle landing to simulate the egress scenario and confirm the ground-based results.

To date, 17 astronauts have participated in DSO 331 Emergency Egress study. Due to post flight conditions (i.e., technical problems or medical inadvisability of participation), 12 astronauts have completed both pre- and postflight testing. Approximately 60% of these subjects were unable to complete the testing as originally stipulated (i.e., walk at 3.5 mph for 5 min). At this time, data on helmet CO₂ buildup, skin temperature, video gait analysis, and respiratory gases are being analyzed. This study is manifested on several future missions in 1998 and is approximately 50% complete.

A complementary ground-based study examining the same factors in a more controlled environment has been completed for the Launch-Entry Suit (LES) using a non-conformal helmet. This study examined the buildup of CO₂, thermal load, and metabolic cost of walking 3.5 mph for 5 min in the LES at 4 different g-suit pressures (0.0, 0.5, 1.0, and 1.5 psi). In all, 12 subjects have completed this study. Less than 60% of the subjects could complete the 5 min walk at g-suit levels above 1.0 psi. In the future, a similar study using the Advanced Crew Escape Suit (ACES) will be performed since preliminary data suggest that subjects are more comfortable in the

ACES suit and are more likely to complete the 5 min walk at higher g-suit levels. This is important since the ACES suit is more commonly worn by the astronaut corps now than the LES suit. A bed rest study is anticipated in which subjects would perform the LES or ACES suit walk as described above after spending 2-3 weeks in head-down bed rest (simulated microgravity) in order to evaluate the ability to complete testing after bed rest deconditioning; this study would thus simulate an emergency egress after several weeks exposure to microgravity only in the more controlled environment of the lab.

This is an operationally-oriented study to assess the feasibility of successful emergency egress. The results of this study will directly benefit crew members by identifying the limiting factors to successful egress (leg fatigue, CO₂ build-up in helmet, overheating) and by offering recommendations to address such limitations (altered helmet or G-suit design, recommended G-suit inflation for egress, leg strength training, additional pre-cooling). Earth-based benefits from this study might relate to applications for workers who must wear protective clothing (e.g., nuclear plant workers), protective breathing systems (e.g., firefighters), or anti-G garments (e.g., high performance aircraft pilots).

Effect of Microgravity on Vascular Cell Function

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-08-17-72

Solicitation: not available

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 1

Post-Doctoral Associates: 2

FY 1997 Funding: not available

Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Task Description:

Information on the effects of microgravity on normal and pathologic vessel wall function is limited and is primarily from studies of vascularized tissues in rats flown in the Cosmos biosatellites. Invagination of endothelium into the lumen of capillaries of the heart has been reported, suggesting pathological activation of endothelial cells (EC). Injured and discontinuous endothelium in rat skeletal muscles have also been observed. Conditions of microgravity may likewise induce activation of macrophages. Infiltration of macrophages into muscle tissues in space-flown rats has been described and resident macrophages shown to be enlarged and activated. Microgravity also activates cultured monocytic cells; mouse peritoneal macrophages during parabolic flight produce four-fold more superoxide than cells not exposed to microgravity. These altered processes, namely, EC injury or dysfunction and macrophage infiltration and activation, together with lipid accumulation and smooth muscle cell (SMC) migration and proliferation, are hallmarks of atherosclerotic lesion formation. We propose that under conditions of microgravity, the functions of cells in arterial vessels are similarly altered, and that these alterations may accelerate the onset of atherosclerosis during long-term space flight. In particular, we propose that conditions of microgravity enhance the pro-oxidant activity of macrophages thereby increasing their capacity to modify low density lipoprotein (LDL) to its putatively atherogenic form, i.e., oxidized LDL. We further propose that microgravity induces dysfunction of the endothelium either by direct injury to EC, or by diminishing the migratory wound-healing responses of EC. These hypotheses will be tested using a cell culture system featuring alternating orientation to simulate microgravity by neutralization of the gravity vector. In particular, we will pursue the following Specific Aims: 1) Determine the effects of simulated microgravity on vascular cell pro-oxidant activity. We will measure the effect of simulated microgravity on oxidation of LDL by activated monocytic U937 cells. We will also measure the cellular release of factors involved in oxidation, namely, superoxide and ceruloplasmin; and 2) Determine the effect of simulated microgravity on endothelial cell motility and its regulatory signaling pathways. We will evaluate the effect of microgravity on wound-induced aortic EC movement and its regulatory signal transduction pathways. We will focus on basic fibroblast growth factor (FGF)-mediated motility and a newly identified G-protein-mediated phospholipase A2 (PLA2) pathway required for EC movement. Successful completion of these Specific Aims will provide important information on the influence of microgravity on key vascular cell processes. These results will be important for the design of *in vitro* investigations under conditions of true microgravity, and may provide insights into potential vessel wall pathologies in animals and humans exposed to conditions of microgravity during prolonged periods.

We have assembled a system to simulate microgravity using slow rotation to “neutralize” the gravity vector. Adherent bovine aortic endothelial cells (EC) are grown to confluence in shallow tissue culture wells. The wells are filled to the rim with medium to minimize mixing during rotation, and are tightly sealed. In the “simulated microgravity” treatment, the culture dish is rotated about a horizontal axis; the time-averaged gravity vector, from the perspective of the cells, is zero. A stationary control is used for comparison to usual culture conditions; the gravity vector is constant at 1-G and directed from the luminal aspect to the basal aspect. A second control consists of “upside-down” cells; the gravity vector is constant, directed from the basal aspect to the luminal aspect. This control group is used to examine if altered responses in the simulated microgravity group are simply due to the time that the cells spent upside rather than to neutralization of the gravity vector. A “Z-axis rotation” control is used to determine whether changes found in the simulated microgravity group are due to centrifugal force or mixing; the gravity vector is constant in this case and directed parallel to the cell length.

During the previous year, we extended our studies on the effect of simulated microgravity on serum-stimulated EC movement. EC migration is a critical and initiating step in angiogenesis, an important process during development, wound-healing, and tumorigenesis. Aortic EC were grown to confluence in plastic tissue culture dishes and a razor wound was made to demarcate the position of cells at time zero. Triplicate wells were subjected to simulated microgravity for 24 h and the cultures fixed and stained for quantitation of cell movement by computer-assisted image analysis. Simulated microgravity treatment did not alter the basal (unstimulated) rate of EC movement, however, serum-stimulated movement was markedly diminished compared to stationary/upright and Z-axis rotation controls. Quantitation of these results as number of migrating cells (or mean distance traveled) showed that simulated microgravity-treatment decreased serum-stimulated migration by about 50% (compared to unstimulated cells). To show that the inhibition was not due to cell injury, the rate of total protein synthesis was measured by incorporation of [3H]leucine into protein. The results were very surprising to us; protein synthesis was substantially increased (up to a doubling was seen in some experiments) by exposure of cells to simulated microgravity. This shows that cell viability was not an issue, but it also suggests that a major alteration in cellular processes must be occurring. Metabolic labeling of EC by [35S]methionine, followed by SDS-PAGE and autoradiography, showed that the increase was not due to an increase in synthesis of a few major proteins, but rather was the result of a similar increase in all detectable proteins. We have begun to examine the mechanism by which simulated microgravity alters cell migration and are focusing on nuclear localization and morphology, and on cytoskeletal rearrangements as measured by confocal microscopy. Our overall conclusion to date is that exposure of EC to simulated microgravity markedly influences multiple biochemical and physiological processes of these cells.

Most investigations of the influence of microgravity on human and animal physiology have been limited to experiments of short duration and have thus focused on acutely altered processes. Future prolonged space flights will provide an opportunity to investigate the effects of microgravity on long-term physiological processes, e.g., development and slow-onset diseases. Information gained from Earth-bound studies can contribute to the success of these flight studies since they may suggest processes particularly worthy of study due to their unusual susceptibility to microgravity or their critical importance to astronaut health. Studies of astronauts and animals returning from space show rapid alterations in bone and muscle physiology as well as compromised immunological function. Although there have not been investigations focused on the effects of microgravity on normal and pathologic vessel wall function, some information is available, primarily from studies of vascularized tissues in rats flown in the Cosmos biosatellites. Invagination of endothelium into the lumen of capillaries of the heart has been reported, suggesting pathological activation of endothelial cells (EC). Injured and discontinuous endothelium in rat skeletal muscles have also been observed. Conditions of microgravity may likewise induce activation of macrophages. Infiltration of macrophages into muscle tissues in space-flown rats has been described and resident macrophages shown to be enlarged and activated. Microgravity also activates cultured monocytic cells; mouse peritoneal macrophages during parabolic flight produce four-fold more superoxide than cells not exposed to microgravity.

Simulation of microgravity affords unique opportunities for novel findings in cell biology. Successful completion of these studies will provide important information on the influence of microgravity on key vascular cell processes. These results will aid in the design of *in vitro* studies under conditions of true microgravity, and

may provide insights into potential vessel wall pathologies in animals and humans exposed to conditions of microgravity during prolonged periods.

FY97 Publications, Presentations, and Other Accomplishments:

Mazumder, B., Mukhopadhyay, C.K., Prok, A., Cathcart, M.K., and Fox, P.L. "Induction of ceruloplasmin synthesis by IFN-g in human monocytic cells." *J. Immunol.*, 159, 1938-1944 (1997).

Mukhopadhyay, C.K., Attieh, Z.K., and Fox, P.L. "Role of ceruloplasmin in cellular iron uptake." *Science* (In Press).

Mukhopadhyay, C.K., Mazumder, B., Lindley, P.F., and Fox, P.L. "Identification of the prooxidant site of human ceruloplasmin: A model for oxidative damage by copper bound to protein surfaces." *Proc. Natl. Acad. Sci. USA* 94: 11546-51 (1997).

Pitsch, R.J., Minion, D.J., Gorman, M.L., vanAalst, J., Fox, P.L., and Graham, L.M. "Platelet-derived growth factor production by cells from Dacron grafts implanted in a canine model." *J. Vasc. Surg.*, 26, 70-78 (1997).

Effects of Artificial Gravity: Central Nervous System Neurochemical Studies

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Funding:

UPN/Project Identification: 199-16-17-14

Solicitation: not available

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Students Funded Under Research: 6

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

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Task Description:

The purpose of this research is to determine neurochemical and morphological changes in the central nervous system of rats following chronic exposure to altered gravity (hypergravity) or to conditions which simulate some of the effects of altered gravity (i.e., hindlimb suspension). The research is based on the hypothesis that chronic exposure to conditions of altered gravity produces changes in sensory feedback related to the control of posture and locomotion. In addition, it is assumed that these changes lead to alterations in chemical activity of neurons and glial cells in the somatosensory cortex. Rats were subjected to hypergravity for up to 14 days and immunocytochemistry was used to study changes in GABA in the hindlimb representation of the motor cortex.

A desktop computer-based method for quantitative assessment of the area occupied by immunoreactive terminals in close apposition to nerve cells using image analysis was developed. This method provides an objective means of measuring area by determining the total area of immunoreactive terminals in light microscopic sections. The difficulties of labeling intensity, size, shape and numerical density of terminals are avoided with this method. Sensitivity of the method was illustrated by application to the analysis of GABA-immunoreactivity associated with pyramidal cells in layer V of the hindlimb representation in animals chronically exposed to hypergravity. Additional studies demonstrated that the area of GABA-IR asomatic terminals apposed to pyramidal cells was reduced in rats exposed to hypergravity as compared with control rats which were exposed either to rotation alone or to vivarium housing conditions. This reduction was interpreted as due to changes in sensory feedback from muscle and it was proposed that this change in GABAergic cells is associated with reprogramming of motor outputs to achieve effective movement control (i.e., adaptation) in the new environmental conditions. Because GABA-IR is latered from chronic exposure to both simulated microgravity (i.e., following hindlimb suspension as reported in FY96), it was suggested that the GABAergic system may be importantly involved as part of the basic adaptive mechanism in motor control.

The central objective of this research is to expand understanding of how gravity affects neuromuscular systems that control posture and gait. The project uses an approach of integrated study in which molecular changes in the neuromuscular system are related to the development of effective motor control. The research will characterize neurochemical changes that occur in sensory and motor systems and relate those changes to motor

behavior as animals adapt to altered gravity. Thus, this research will identify changes in central and peripheral neuromuscular mechanisms as motor control is reestablished after disruption by exposure to hypergravity. Improved understanding of the relationship of mechanisms of "plasticity" in the neuromuscular system to motor control will suggest mechanisms that could contribute to alterations in motor control during and following space flight. Findings from this research also may have clinical applications. Motor control is disrupted by miscellaneous injuries (e.g., spinal trauma, blunt head injury, stroke, damage to the vestibular system) and disease states (e.g., multiple sclerosis, ALS) that affect various components of the neuromuscular system. Findings from this integrated approach to studying molecular and functional alterations in the neuromuscular system will suggest various neuromuscular structures (e.g., motor neurons, cortex, muscle receptors) and neurotransmitters (e.g., GABA) that may contribute to the development of effective motor control as the neuromuscular system reacts to injury or disease.

FY97 Publications, Presentations, and Other Accomplishments:

D'Amelio, F., Wu, L.C., Fox, R.A., Daunton, N., Corcoran, M.L., and Polyakov, I. "Hypergravity exposure decreases GABA immunoreactivity in axon terminals contacting pyramidal cells in the rat somatosensory cortex: A quantitative immunocytochemical image analysis." *J. Neurosci. Res.* (In Press).

Wu, L.C., D'Amelio, F., Fox, R.A., Polyakov, I., and Daunton, N. "Light microscopic image analysis system to quantify immunoreactive terminal area apposed to nerve cells." *J. Neurosci. Meth.*, 89-96 (1997).

Respiratory Afferents and the Control of Breathing

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 5 P01 HL40369-08 (NIH) Solicitation: not available
Initial Funding Date: 1989 Expiration: 1998
Students Funded Under Research: 0 Post-Doctoral Associates: 0
FY 1997 Funding: \$ 118,152
Joint Agency Participation: NIH/National Heart, Lung, and Blood Institute

Task Description:

This project is a supplement to the Program Project Grant (PPG) "Respiratory Afferents and Control of Breathing" (HL 40369: Donald Frazier, Principal Investigator). It is a direct extension of the principles and procedures developed in Project 2 "Integrative Reflex Dynamics in Respiratory Cycle Control" (Eugene Bruce, project Principal Investigator) of the PPG. The ongoing studies of Project 2 involve only acutely tracheotomized rats and rats with intact upper airway (UAW), whereas the new studies will evaluate many of the same responses and mechanisms in rats subjected to chronic tracheotomy. The ongoing studies will provide data for comparison with the proposed studies. As in this task, a major focus of studies in Project 2 is to define UAW muscle contributions to, and UAW-related mechanisms underlying, responses of the respiratory pattern to neural and chemoreflex stimuli (both mean responses and changes in breath to breath variability of tidal volume, breath timing, and chest wall and upper airway EMGs). These objectives are expressed explicitly in two of the 5 specific aims of Project 2, which encompass approximately 50% of the total work. The other specific aims of Project 2 address the responses of various types of pulmonary vagal afferents to lung deflation in the rat, central projections of vagal slowly-adapting deflation receptors, and the interactions of chemical drive, pulmonary afferents, and UAW afferents on breathing pattern variability when the upper airway is bypassed acutely. This last aim also is addressed in the studies of this supplement. Thus, this research directly complements much of current Project 2 by determining the effects of chronic tracheotomy on the responses and respiratory control mechanisms being studied in Project 2.

The study of the respiratory consequences of chronic changes in the upper airway is a logical and desirable enhancement of the PPG project since the health-related application of basic research often involves chronic situations which are not addressed directly in research studies. Furthermore, there is evidence (see Background) that control of upper airway muscles is indeed different in these chronic situations than in the acute studies of Project 2. Most of the experimental procedures to be used in the animal studies are identical to those being used in Project 2 in order that such differences can be identified.

This supplemental project also includes human studies in which both acute and long-term changes in upper airway mechanics are expected due to gravitational effects and vascular fluid shifts. Evaluating the applicability of findings from animal studies to humans is always a necessary step which, however, is complicated by the inability to impose the degree of experimental control that one utilizes in animal studies. Instead of

tracheotomy, we will consider gravitational effects and vascular fluid shifts which should alter upper airway resistance. The studies will both confirm this effect on the upper airway in humans and address relatively long-term (48 hours) adaptations to this change in resistive load on breathing. These studies are possible because of an ongoing NASA-sponsored study of cardiovascular responses to simulated weightlessness which includes a head-down bed rest protocol in a Clinical Research Center and MRI analyses of the volumes of thoracic great vessels. We will use head-down bed rest to produce acute and chronic gravitational changes in upper airway conformation and shifts in vascular volumes toward the thorax, head, and neck. Also, because MRI procedures already are incorporated into those studies, we are able to include imaging of the upper airway as well. One possible application of these proposed studies is to astronauts returning from prolonged missions in microgravity and future studies in true microgravity would extend both the ongoing and proposed studies. However, the studies also should be relevant to restoring the natural airway in subjects undergoing long-term tracheal intubation and to understanding upper airway function in diseases which produce chronic alteration of upper airway resistance.

Circadian Rhythms in Rhesus: Gravity, Light & Gender

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Funding:

UPN/Project Identification: 199-18-17-18

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 5

Post-Doctoral Associates: 0

FY 1997 Funding: \$166,592

Task Description:

This project will examine the influences of gravity and light on the circadian system (CTS) in unrestrained male and female rhesus macaques (*Macaca mulatta*). The CTS coordinates the temporal aspects of physiology and behavior. The light-dark cycle is the major time cue used by the CTS. Disruptions in circadian timing adversely affect an organism's ability to respond to environmental challenges, decrease performance, and contribute to psychological disorders. Circadian timing is altered under both the microgravity of space flight and hyperdynamic fields produced by centrifugation. In addition, prolonged exposure to a lighting environment similar to that currently used on the shuttle and planned for the international space station can produce debilities in individuals on the ground. The experiments will determine the effects of a hyperdynamic environment on the CTS in rhesus monkeys and characterize any gender differences in CTS function.

During FY 97, cohorts of 8 male and 8 female rhesus were trained to use the Psychomotor Test System (PTS) developed at the University of Georgia. All dietary requirements are met through the use of PTS. In all our studies, PTS was available to the animal whenever the lights were on.

Two initial studies were conducted at the California Regional Primate Research Center using the male subjects. In the first study, drinking and PTS performance were recorded under conditions of LD 16:8 (16 hours of light followed by 8 hours of darkness) for two weeks followed by 6 weeks of constant light (LL). Under the light-dark cycle, the animals displayed entrained circadian rhythms with a period of 24.0 hours; in LL the period of the rhythms shortened to an average of 23.6 hours. Homeostatic levels of PTS trials were maintained, but the amplitude of the rhythm was smaller in LL than LD. In the second study, the base LD 16:8 cycle was advanced and delayed by 6 hours. Both the advance and the delay resulted in a transient desynchronization (similar to jet lag) and similar amounts of time were required to achieve reentrainment in both conditions.

We also performed 2-G pilot study with the male rhesus as subjects. These data showed an initial suppression of circadian rhythmicity at the onset of 2-G followed by a gradual return of rhythms.

We have tracked the reproductive cyclicity of the female subjects over the past year. Daily urine samples have been collected and are undergoing analysis for estrone and progesterone conjugates. When the cyclicity is confirmed, we will begin using the females in our 1-G and 2-G studies.

We know from previous space research that exposure to space flight affects the circadian rhythms of organisms ranging from unicells to primates. Different rhythms do not respond in the same fashion, producing an internal desynchronization between the various circadian rhythms. Desynchronization between internal rhythms may be linked to reduced capabilities in the performance of simple tasks and to psychological abnormalities. An absence of external time cues has been shown to interfere with normal thermoregulation in the squirrel monkey. In addition, several sleep and psychological disorders have close relationships with circadian dysfunction.

This program is designed to examine the effects of exposure to a hyperdynamic environment on rhythms of various functions and to elucidate any differences in the responses of the two genders. There is a preponderance of women among those treated for psychological disorders, including those linked to circadian dysfunction. This has been attributed to various physiological, psychological, and sociological differences, but no innate underlying cause has yet been proved.

Women now form a substantial part of the space research program and are frequent space travelers. There is an additional concern of body calcium levels. Women are at greater risk for calcium loss from bone through osteoporosis and start with a smaller base of bone calcium than do males. The bone calcium loss in space flight arouses additional concerns for female astronauts.

FY97 Publications, Presentations, and Other Accomplishments:

Barger, L.K., Hoban-Higgins, T.M., and Fuller, C.A. "Effects of lighting conditions on circadian rhythms of performance and drinking in rhesus monkeys." *FASEB J.*, 11(3), A636 (1997).

Intercompartmental Fluid Shifts in Response to Postural and Gravitational Forces

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Funding:

UPN/Project Identification: 199-14-17-03

Solicitation: not available

Initial Funding Date: 1993

Expiration: 1997

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

No additional information was supplied by the principal investigator.

Neurocognitive Function Test for Space Flight Crew Members

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Funding:

UPN/Project Identification: 199-16-17-15

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$196,514

Task Description:

We propose to develop an efficient, sensitive, reliable, and cost-effective means for assessing changes over time in individual crew members of several fundamental higher cognitive brain functions. The Neurocognitive Function Test (NFT) would be useful for studying changes in these functions during long-duration space missions, and would also have dual-use application in ground-based studies of operational fatigue, biological rhythms, medications, or environmental stressors. Unique to the NFT will be the ability to quickly measure how multivariate combinations of behavioral, physiologic, and neuro-physiologic indices of attentional and working memory functions and fine perceptuomotor control change in an individual crew member over time. The basic research to be performed will include pilot studies to determine stability and retest reliability of behavioral, physiologic, and neurophysiologic measures, followed by a laboratory experiment to determine the test's sensitivity to operational fatigue and other sources of impairment, and its ability to validly predict the potential for compromised performance. As a final component of the project, we will perform a small study in which the NFT is used to measure operational fatigue in a more realistic context. This latter component of the project will be completed in collaboration with Dr. Mark Rosekind of the NASA Ames Fatigue Countermeasures Program. In addition to research applications, the NFT could ultimately prove to be a sensitive test of work readiness in a wide range of military and civilian work environments.

To date, substantial progress has been achieved toward the objectives of this project. Progress has been made in several different areas. First, we have performed further analyses on a dataset collected in a pilot study performed before the start of this project. That pilot study compared EEG and behavioral measures while nine subjects performed computer-based tasks during an alert baseline state, a mildly intoxicated state, and a fatigued state. These analyses helped us to evaluate the relative utility of different tasks for inclusion in the test battery, and to identify candidate features of the EEG to serve as markers of impaired cognition. Current work in progress involving this dataset includes further analyses needed to develop improved indices of drowsiness, and the preparation of a manuscript to report the associated results.

Second, based on these preliminary results and on review of the relevant literature, we refined our test battery. It now includes a test of working memory that we have studied intensively in our laboratory, and the Psychomotor Vigilance Task (PVT). The PVT has been used extensively by our colleagues Dr. Mark Rosekind (Chief, Aviations Operation Branch, NASA Ames Research Center, and Principal Investigator of the Fatigue Countermeasures Program) and Dr. David Dinges (Director, Unit for Experimental Psychiatry, Division of Sleep

and Chronobiology (University of Pennsylvania) in behavioral studies of fatigue in operational environments. We initially performed small pilot tests with this battery, and have recently completed data collection from a formal experiment with 20 subjects in which we are examining the test-retest reliability of behavioral and EEG indices in the normal waking state. Analyses of this dataset has been completed. We are also in the piloting stage of a second formal study to determine the sensitivity of these measures to changes in mental function that could impair performance, using the effects of operational fatigue, low doses of alcohol, and OTC antihistamines as model forms of impairment.

Finally, as part of the planned study, we hope to determine the predictive or criterion validity of the NFT by examining whether it predicts impairment on a post-test task that simulates activities in a high-performance operational environment. Towards this end we have obtained a relevant simulation task, the NASA Multi-Attribute Task battery (Comstock and Arnegard, 1992). This battery incorporates tasks analogous to activities that aircraft crewmembers perform in flight, and it has been used extensively in research on workload and adaptive automation. This battery has been installed in our laboratory and we have begun to conduct small pilot studies with it in preparation for its use in the planned formal study.

In addition to its use in NASA research studies on the effects of long-duration space flight on higher brain function, the potential benefits of the NFT could form the basis of a dual use technology for assessing "Readiness-To-Perform" in military and civilian work environments. That is, the NFT could help reduce the incidence of tragic accidents related to human error. It could serve to detect impaired attention and alertness (resulting from fatigue, hangover, illness, or other debilitating conditions) in personnel critical for chemical and power plant operation, crisis management, aviation, emergency medicine, and military combat. There is a great societal need and a viable market for such a device.

FY97 Publications, Presentations, and Other Accomplishments:

Gevins, A. (invited lecturer) 50th Annual Meeting of the US Western EEG Society, Los Angeles (February, 1997).

Gevins, A. (invited speaker) Pan Pacific Conference on Brain Topography, Tokyo (April, 1997).

Gevins, A. (invited speaker) International Epilepsy Society Congress, Dublin (June, 1997).

Gevins, A. (keynote speaker) International Society for Brain Electromagnetic Topography, Rio (October, 1996).

Gevins, A. (participant) Air Force New World Vistas "People" Program Kickoff, San Antonio (May, 1997).

Gevins, A., Smith, M.E., Leong, H., McEvoy, L., Whitfield, S., Du, R., and Rush, G. "Monitoring working memory load during computer-based tasks with EEG pattern recognition." *Human Factors* (In Press).

Gevins, A., Smith, M.E., McEvoy, L., and Yu, D. "High resolution EEG mapping of cortical activation related to working memory: Effects of task difficulty, type of processing, and practice." *Cerebral Cortex*, 7, 374-385 (1997).

Le, J., Lu, M., Pellouchoud, E., and Gevins, A. "A rapid method for determining standard 10/10 electrode positions for high resolution EEG studies." *Electroencephalography Clin. Neurophysiol.* (In Press).

Pellouchoud, E., Leong, H., and Gevins, A. "Implications of electrolyte dispersion for high-resolution EEG methods." *Electroencephalography Clin. Neurophysiol.*, 102, 261-263 (1997).

Physiological Monitoring of Mental Workload

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Funding:

UPN/Project Identification: 199-06-17-08

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 1999

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$100,000

Task Description:

A practical means of measuring the mental workload imposed by a task would be useful in a variety of NASA applications. In human-machine interface design, it would provide an objective means for determining whether one interface imposed less mental workload than another. In training personnel for IVA and EVA tasks, it would provide an index of the relative difficulty of component sub-tasks, helping to identify those which require further practice. Current measurements of mental workload (e.g., a priori task classifications, subjective ratings, performance data) are not sufficient for this purpose. In a recent study we found that weighted combinations of physiological measures (including brain, scalp muscle, and eye-signals) were highly sensitive to manipulations of the mental load imposed by a task; using neural networks, mental workload variations could be classified accurately and reliably. Such measures are well-suited for use in operational environments since they can be collected unobtrusively and in real-time. We thus propose to further develop this technology by extending it to more realistic and mission-relevant tasks, including a complex divided attention task that approximates a display panel multi-tasking environment, and a realistic training simulator in collaboration with Dr. Mark Rosekind at the Aviation Operations Branch of the NASA Ames Research Center.

Funding for this project did not commence until the end of FY97. Substantive work on the project is beginning in FY98.

In addition to its use in NASA research studies on mental workload, the potential benefits of the results of this study lie in the development of an effective means for assessing mental workload in a wide range of military and civilian work environments. That is, the further development of such technology is critical for optimizing the human-system interface: it could provide crucial missing information during the design and evaluation of complex systems; it could improve the efficiency of personnel selection and training efforts; it could help assess fitness-for-duty; and it could make it possible to unobtrusively monitor the cognitive load of both individual personnel and work groups and thus aid in the evaluation of work practices.

FY97 Publications, Presentations, and Other Accomplishments:

Gevins, A. (invited lecturer). 50th Annual Meeting of the US Western EEG Society, Los Angeles (February, 1997).

Gevins, A. (invited speaker). Pan Pacific Conference on Brain Topography, Tokyo (April, 1997).

Gevins, A. (invited speaker). International Epilepsy Society Congress, Dublin (June, 1997).

Gevins, A. (keynote speaker). International Society for Brain Electromagnetic Topography, Rio (October, 1996).

Gevins, A. (participant). Air Force New World Vistas "People" Program Kickoff, San Antonio (May, 1997).

Gevins, A., Smith, M.E., Leong, H., McEvoy, L., Whitfield, S., Du, R., and Rush, G. "Monitoring working memory load during computer-based tasks with EEG pattern recognition." *Human Factors* (In Press).

Gevins, A., Smith, M.E., McEvoy, L., and Yu, D. "High resolution EEG mapping of cortical activation related to working memory: Effects of task difficulty, type of processing, and practice." *Cerebral Cortex*, 7, 374-385 (1997).

Le, J., Lu, M., Pellouchoud, E., and Gevins, A. "A rapid method for determining standard 10/10 electrode positions for high resolution EEG studies." *Electroencephalography Clin. Neurophysiol.* (In Press).

Pellouchoud, E., Leong, H., and Gevins, A. "Implications of electrolyte dispersion for high-resolution EEG methods." *Electroencephalography Clin. Neurophysiol.*, 102, 26-263 (1997).

Role of Integrins in Mechanical Loading of Osteoblasts

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Funding:

UPN/Project Identification: 199-26-17-15

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 159,566

Task Description:

Mechanical forces generated by gravity, weightbearing, and muscle contraction play a key role in the genesis and maintenance of skeletal structure. Increased mechanical loading caused by exercise stimulates osteoblasts resulting in increased bone formation and accretion of skeletal mass. Conversely, astronauts exposed to prolonged space flight suffer from site-selective osteopenia, which has been shown in growing rats to result from reduced bone formation by osteoblasts. The reduction in bone formation appears to be caused by defects at several stages of osteoblast differentiation, including proliferation, matrix production, and mineralization. The molecular mechanisms that mediate changes in osteoblast activity in response to altered patterns of skeletal loading are not known, and a better understanding of these processes may be essential for developing effective treatment strategies to prevent disuse osteoporosis.

The long-term goal of our collaborative research program is to understand how the extracellular matrix (ECM) and cell adhesion proteins, integrins, interact to mediate the response of osteoblasts and their progenitors to mechanical loading. We propose to test elements of the following speculative model. Mechanical force distorts the ECM that surrounds osteoprogenitors and osteoblasts, resulting in activation of their integrin receptors on their cell surface which link specific matrix ligands in the extracellular space to cytoskeletal elements inside the cell. Mechanical signaling either through integrins or through other mechanoreceptors such as ion channels regulates the expression of genes involved in proliferation and differentiation of osteoblasts and their progenitors. Since changes in integrin expression and activity help mediate specific processes of progressive osteoblast differentiation during embryogenesis (the subject of a separate NIH project, C. Damsky, P.I.), we predict that mechanical loading also regulates integrin expression and function downstream of the initial signaling events. Thus, we suggest that integrin/ECM interactions are crucial both for the perception of mechanical signals and in mediating the cellular responses to such stimuli.

We propose to: 1) determine if hindlimb unloading of growing rats *in vivo* alters the expression of integrins in bone; 2) determine how changes in mechanical loading affect integrin expression during progressive osteoblast differentiation *in vitro* using primary rat osteoblasts exposed to stretch; and 3) test the hypothesis that specific integrin-ECM interactions mediate mechanical stretch-induced changes in osteoblast function *in vitro*.

Progress was made in three general areas:

1) Extend previous experiments to identify the functional relevance of ECM ligands and integrin receptors.

To evaluate the functional role of specific ECM ligands and integrin receptors, two approaches have been employed using the primary fetal rat calvarial cell culture. In brief, cells isolated from 21-day old fetal rat calvarial are grown in culture, and following the addition of serum, ascorbate and β -glycerophosphate, produce mineralized nodules that histologically resemble woven bone. The two approaches used to evaluate functional aspects of the osteoblast employ both genetic (adenovirus infection) and function-perturbing (antibody) methods.

Identify integrin receptors involved in osteoblast differentiation. We previously reported that cellular interactions with fibronectin (FN) appear to be required for progressive osteoblast differentiation since the addition of function-perturbing antibodies, FN fragments that correspond to the central cell binding domain and RGD peptides, inhibit osteoblast differentiation and morphogenesis of mineralized nodules (Moursi, *J. Cell Sci.*, 109:1369-1380, 1996). We extended these studies this year by examining the ability of function-perturbing antibodies against specific integrins to inhibit differentiation. We report that interfering with the interactions of $\alpha 5 \beta 1$, a specific FN receptor, suppresses mineralized nodule morphogenesis and osteoblast differentiation. In addition, $\alpha 3 \beta 1$ and $\alpha 8 \beta 1$ integrins, which recognize FN as well as other ligands present in bone ECM, also regulate osteogenesis. In contrast, perturbing the interactions of the FN receptors $\alpha V \beta 3$ and $\alpha V \beta 5$ did not block mineralized nodule formation. Thus, interactions between ECM ligands, including FN and specific integrin receptors, are required for osteoblast differentiation.

Role of FN as survival factor for mature osteoblasts. To determine if FN also plays an important role in the function of mature osteoblasts as well as during differentiation, osteoblasts that had already formed mineralized nodules *in vitro* were treated with FN antagonists FN antibodies (FNAb) caused >95% of the cells in mature cultures to display characteristic features of apoptosis within 24 h (nuclear condensation, apoptotic body formation, DNA laddering). Cells appeared to acquire sensitivity to FNAb-induced apoptosis as a consequence of differentiation, since FNAb failed to kill immature cells and the first cells killed by FNAb were those associated with mature nodules. Intact plasma FN, as well as fragments corresponding to the amino-terminal, cell-binding, and carboxy-terminal domains of FN, independently induced apoptosis of mature (13d), but not immature (4d), osteoblasts. Thus FN appears to function in the mature, but not the immature, ECM to sustain osteoblast survival. Finally, transforming growth factor- $\beta 1$ partially protected cells from the apoptotic effects of FNAb, indicating that TGF- β may function in concert with FN to promote osteoblast survival *in vivo*. We conclude that FN functions to promote survival of osteoblasts once they have matured, and that this may contribute to the regulation of bone formation. Future experiments will focus on whether mechanical strain regulates FN-mediated osteoblast survival.

Other ECM components (laminin, tenascin-C, col I). To determine if other ECM components participate in the differentiation of osteoblasts, cells were treated with function-perturbing antibodies. The continuous addition of laminin antibodies to maturing cultures inhibited alkaline phosphatase activity and nodule formation, whereas tenascin-C and SPARC (osteonectin) antibodies did not affect on nodule formation.

2) Evaluate the effects of mechanical strain applied *in vitro* on the expression of specific integrins and ECM ligands. Studies have been initiated recently using Flexcell flexible dishes for both control and mechanically active conditions to generate both compressive and tensile strains on primary osteoblasts. The expression of specific ECM, integrin, and cytoskeletal components in response to mechanical forces (4% maximum deformation, 0.5 cycles/sec) were analyzed using immunocytochemical techniques. We found that consistent changes in the pattern of expression of fibronectin, $\alpha 5$ integrin, and actin are not evident after 2hr, 2d or 4d of strain relative to stationary controls (unpublished results).

Since the mechanical strain provided by the Flexcell system is non-uniform and provides strain levels that greatly exceed physiologic levels, we have initiated a collaboration with scientists and engineers at NASA-Ames Research Center (N. Searby, E. Holmuhamedov, E. Morey-Holton) to develop a novel strain unit that will: (a) load each cell uniformly and physiologically; (b) dynamically load cells in cyclic tension compression; (c)

permit real-time microscopic observations of the cells at a single set-point in each loading cycle; and (d) provide the capability to independently apply fluid shear forces. A prototype loading has been built and loading chambers are being tested to assess biocompatibility. Future experiments testing the role of integrins during *in vitro* mechanical loading of osteoblasts will be performed using this apparatus.

3) Evaluate effects of hindlimb-unloading and reloading on the expression of specific integrins and ECM ligands. Experiments have been initiated to determine if the pattern or level of expression of specific integrins or ECM ligands is altered by *in vivo* hindlimb unloading. Growing rats (6 wks of age) were hindlimb-unloaded for 7d, with controls pair fed. A third group was first hindlimb-unloaded for 7d then removed from suspension and allowed to ambulate normally for two days before sacrifice. Tibiae were dissected free then prepared using various histological methods for immunocytochemical analysis. The contralateral tibiae were removed, periosteal cells isolated by collagenase digestion, then the cells lysed for later analysis of integrin expression by immunoprecipitation techniques. Experiments to analyze other integrin and ECM components that were identified in our function-blocking experiments to regulate osteoblast differentiation or survival (Section IIA) are in progress.

Prolonged space flight or physical inactivity cause disuse osteoporosis, shown in growing rats to be caused by a defect in bone formation by osteoblasts. The molecular mechanisms underlying these processes are not well-understood, and once known, may facilitate the development of effective countermeasures. In addition, results from these studies are expected to contribute new information about how mechanical signals are transduced within the cell, a basic biological process that is not yet fully understood.

FY97 Publications, Presentations, and Other Accomplishments:

Globus, R.K., Doty, S.B., and Damsky, C.H. "Disruption of fibronectin interactions causes differentiation-dependent apoptosis of cultured osteoblasts." (Abstract) *J. Bone Min. Res.*, 3(Suppl.1), S146 (1997).

Globus, R.K., Holmuhamedov, E., Lull, J., Lopez, V., Moursi, A., Doty, S., and Damsky, C. "Fibronectin is a survival factor for mature osteoblasts." (Abstract) *Mol. Biol. Cell*, 5 (Suppl.), 564a (1996).

Globus, R.K. "Fibronectin and osteoblast survival." Gordon Conference on Fibronectin, Integrins, and Related Molecules (1997).

Morey-Holton, E.H. and Globus, R.K. "Skeletal changes during hindlimb unloading of growing rats: A review." (In Press).

Moursi, A.M., Najafi, M., Globus, R.K., and Damsky, C. "Integrin-fibronectin interactions are critical for osteogenesis and osteoblast differentiation.." (Abstract) *J. Dent. Res.*, 76, 296 (1997).

Heart Rate Dynamics During Microgravity Exposure: Data Analysis

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Funding:

UPN/Project Identification: 199-14-17-19
Initial Funding Date: 1994
Students Funded Under Research: 0
FY 1997 Funding: \$91,260

Solicitation: 95-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 0

Task Description:

NASA has prioritized the development of new, efficient, and inexpensive ways to archive, disseminate, and analyze the vast amounts of physiological data obtained during space flight and also during ground-based simulations of microgravity exposure. NASA has also given priority to the investigation of two problems encountered in space flight, both of which influence astronaut behavior and performance: 1) space motion sickness (SMS); and 2) cardiovascular deconditioning, especially during long-term missions. We propose to use spectral and nonlinear analyses of heart rate data as quantitative methods for detecting the presence of these problems, to evaluate potential countermeasures against them, and to study their physiologic mechanisms.

Our objectives are intended to extend our results developed during our current NASA grant (NAG-9-572):

- 1) To compile and analyze digitized databases (pre-flight, during flight, and post-flight) of continuous ECG recordings from de-identified crew members from U.S. Spacelab Life Sciences and Shuttle missions and Russian Mir missions and to store these databases on compact discs (CD-ROM format) to facilitate distribution and retrieval.
- 2) To quantify the loss of complex heart rate variability as a potentially useful index of cardiac deconditioning during space flight, and during microgravity simulations with bedrest, in order to assess the effects of countermeasures such as LBNP and exercise.
- 3) To correlate the distinctive low frequency ($< .01$ Hz) heart rate oscillations observed during space flight with (a) subjective motion sickness symptoms, (b) activity level, and (c) a respiratory signal derived from the Holter ECG.
- 4) To further develop a new nonlinear model of heart rate control and to incorporate gravitational effects to understand the mechanism of the observed dynamics.

These extensive, ongoing data analysis studies of spaceflight and microgravity simulation are being conducted in collaboration with NASA scientists at Johnson Space Center and the Ames Research Center, and with Russian space scientists at the Moscow Center for Biomedical Research.

During the past fiscal year, we have made substantial progress with respect to our specific aims. In particular, we have:

- 1) Continued and extended our collaboration with Professor Roman Baevsky of the Russian Space Program to analyze pre-flight, in-flight, and post-flight Mir data. Dr. Goldberger met with him in Washington, D.C. on June 10, 1997 to arrange for transfer of new data files for joint analysis of cosmonaut Holter monitor data.
- 2) Undertaken the analysis of space flight data with Dr. Jan Yelle, NASA-JSC, obtained as part of a joint Russian/American study. We have performed detailed clinical and time series analysis and are preparing a scientific report with Dr. Yelle and her colleagues.
- 3) Analyzed heart rate responses to lower body negative pressure (LBNP) before and after space flight on US Shuttle crew member in conjunction with Suzanne Fortney, Ph.D., NASA/JSC. These studies revealed a loss of nonlinear heart rate complexity after space flight, confirming and extending earlier work by our laboratories.
- 4) Dr. Goldberger was an invited speaker at the "Advanced Technologies for Gravitational Biology Symposium," January 15-17, 1997, NASA/Ames Research Center. He presented a talk on "Heart Rate and Related Physiological Data Acquisition, Storage, and Analysis: Five Future Imperatives for Success." The past studies of cardiac dynamics in microgravity have been severely hampered by incomplete data acquisition, lack of correlative information, and problems with data storage and retrieval. This talk reviewed these problems and proposed five guiding principles for the Space Station and related explorations, including a major new proposal for creation of a NASA physiologic data bank ("PHYSBank"), to facilitate collaboration, obviate reduplication, and preserve invaluable data.
- 5) Developed new techniques for analysis and modeling of complex, nonstationary heart rate time series (see publications).
- 6) Developed new techniques for analysis of human gait stability and instability using techniques initially developed for heart rate analysis. These studies may have important implications for assessing neuromuscular stability of crew members during and after space flight, as well as "spin-off" applications to clinical neurology (see publications).

This research project seeks to understand two important conditions: space motion sickness and deconditioning associated with bedrest and microgravity. This research is directed at developing new ways of detecting and monitoring these conditions. These techniques promise to have general applications to clinical monitoring and to detecting patients at high risk of cardiopulmonary instability including life-threatening conditions. Furthermore, this work has led to new techniques for monitoring human gait in health and disease. This work has also led, as a spin-off, to new techniques for analyzing coding vs. non-coding DNA.

FY97 Publications, Presentations, and Other Accomplishments:

Goldberger, A.L. "Electrocardiography" in "Harrison's Principles of Internal Medicine." Edited by: Fauci, A.S. et al, 14th Edition. McGraw-Hill, pp 1237-1246 (1997).

Goldberger, A.L. "Fractal variability versus pathologic periodicity: Complexity loss and stereotypy in disease." *Perspectives Biol. & Med.*, 40:4, 543-561 (Summer, 1997).

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Hausdorff, J.M., Edelberg, H.K., Mitchell, S.L., Goldberger, A.L., and Wei, J.E. "Increased gait unsteadiness in community-dwelling elderly fallers." *Arch. Phys. Med. Rehab.*, 78, 278-283 (March, 1997).

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Ho, K.K.L., Moody, G.B., Peng, C-K., Mietus, J.E., Larson, M.G., Levy, D., and Goldberger, A.L. "Predicting survival in heart failure case and control subjects by use of fully automated methods for deriving nonlinear and conventional indices of heart rate dynamics." *Circulation*, 96(3), 842-848 (August 5, 1997).

Iyengar, N., Peng, C-K., Morin, R., Goldberger, A.L., and Lipsitz, L.A. "Age-related alterations in the fractal scaling of cardiac interbeat interval dynamics." *Am. J. Physiol.*, 79, 372-373 (1997).

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Medina, A., Bodick, N., Goldberger, A.L., MacMahon, M., and Lipsitz, L.A. "Effects of central muscarinic-1 receptor stimulation on blood pressure regulation." *Hypertension*, 29(3), 828-834 (March, 1997).

Peng, C-K., Havlin, S., Hausdorff, J.M., Mietus, J.E., Stanley, H.E., and Goldberger, A.L. "Fractal mechanisms and heart rate dynamics: Long-range correlations and their breakdown with disease." *J. Electrocardiol.*, 28, 59-66 (1996).

Schwartz, C.E., Peng, C-K., Lester, N., Daltroy, L.H., and Goldberger, A.L. "Brief report: Self-reported coping behavior and health and disease: Assessment with a card sort game." *Behav. Med.* (In Press).

Stanley, H.E., Buldyrev, S.V., Goldberger, A.L., Havlin, S., Mantegna, R.N., Peng, C-K., and Simons, M. "Scale invariant features of coding and noncoding DNA sequences" in "Fractal Geometry in Biological Systems: An Analytical Approach." Edited by: Iannaccone, P.M. and Khokha, M. CRC Press, Inc. (1996).

Viswanathan, G.M., Peng, C-K., Stanley, H.E., and Goldberger, A.L. "Deviations from uniform power law scaling in nonstationary time series." *Phys. Rev. E.*, 55, 845-849 (1997).

Zimetbaum, P., Kim, K.Y., Ho, K.K.L., Zebede, J., Josephson, M.E., and Goldberger, A.L. "Utility of patient-activated cardiac event recorders in general clinical practice." *Am. J. Cardiol.*, 79, 371-372 (1997).

Exercise Within LBNP to Produce Artificial Gravity

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Funding:

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Responsible NASA Center: Ames Research Center

Task Description:

Calculations suggest that exercise in space to date has lacked sufficient loads to maintain musculoskeletal mass. Lower body negative pressure (LBNP) produces a force at the feet equal to the product of the LBNP and body cross-sectional area at the waist. Supine exercise in 100 mm Hg LBNP improves tolerance to LBNP and produces forces similar to those occurring during upright posture on Earth. Using a broader waist seal, LBNP at 50-60 mm Hg generates normal 1g footward forces. Exercise within LBNP may help prevent deconditioning of astronauts by stressing tissues of the lower body in a manner similar to gravity. Thus, LBNP exercise may provide a safe and effective alternative to centrifugation in terms of cost, mass, volume, and power usage. We hypothesize that supine treadmill exercise during LBNP at one body weight (50-60 mm Hg LBNP) will provide cardiovascular and musculoskeletal loads similar to those experienced while upright in 1g. Also, daily supine treadmill running in a LBNP chamber will maintain aerobic fitness, orthostatic tolerance, and musculoskeletal structure and function during bed rest (simulated microgravity). For bed rest studies, only male subjects will be used because these studies involve a fluid regulation component which is difficult to separate from effects of normal hormonal cycles in females. First, we will compare lower-extremity biomechanics, metabolism, and hemodynamic responses during supine LBNP exercise against 50-60 mm Hg with the same parameters during upright exercise in 1g. Second, bed rest studies will focus on orthostatic tolerance, upright exercise capacity, and leg muscle strength to evaluate efficacy of LBNP exercise. Subjects will experience 6° head-down tilt (HDT)

for 14 days. Subjects will run while supine on a vertical treadmill for 40 min at 50-60 mm Hg LBNP per day throughout the HDT period. Each subject will act as his own control by participating in both exercise and no-exercise bed rest studies. Pre- and post-HDT tests will include orthostatic tolerance, cerebral blood flow, plasma volume, circumferences of body segments, peak oxygen uptake, leg muscle strength, GI function, and gait analyses. We expect that supine LBNP treadmill exercise at one body weight will provide an accurate simulation of cardiovascular and musculoskeletal loads experienced while upright in 1g. We further expect that 40 minutes of supine treadmill running per day in a LBNP chamber will maintain aerobic fitness, orthostatic tolerance, and musculoskeletal structure and function during 14 days of bed rest. The goals and objectives did not change during the course of our two years of NASA support.

The overall goal of this research project is to determine whether treadmill exercise within lower body negative pressure (LBNP) can simulate cardiovascular and musculoskeletal effects of gravity, and in doing so help prevent the physiologic deconditioning normally associated with bed rest and space flight.

The promising results from the five-day bed rest study (Lee *et al.*, 1997) led us to perform a two-week bed rest study with a more strenuous interval exercise protocol (40-80% of peak VO_2) and a more comprehensive battery of pre- and post-bed rest tests. We increased daily LBNP exercise duration to 40 minutes and footward force to 1.0-1.2 body weights, but the 5 min of static LBNP after each LBNP exercise session and the comparison to daily upright exercise training were omitted. Seven male subjects acted as their own controls, such that their responses to two weeks of bed rest with daily supine LBNP treadmill exercise were compared to their responses to two weeks of bed rest with no daily exercise. Their participation in the two bed rest studies was separated by 10 weeks.

Forty min per day of LBNP exercise preserved upright exercise capacity during two weeks of bed rest. Subjects' time to volitional exhaustion during their individualized treadmill tests decreased 1.72 min on average (10%, $p < 0.05$) after bed rest with no daily exercise; daily LBNP exercise during bed rest maintained exercise tolerance time at pre-bed rest levels. Daily LBNP exercise also maintained peak upright VO_2 at pre-bed rest levels (pre-bed rest: 59.5 ± 3.2 ; post-bed rest: 56.4 ± 3.4). Mean peak VO_2 decreased from 57.6 ± 2.6 to 49.8 ± 1.5 ml/min/kg (14%) after bed rest with no exercise. Both respiratory exchange ratio and heart rate were consistently elevated at three sub-maximal running speeds relative to pre-bed rest measurements. Mean post-bed rest ventilation rate was significantly elevated at the highest two sub-maximal running speeds (13% and 17%) relative to pre-bed rest measurements. None of these effects were seen during sub-maximal exercise after bed rest with daily LBNP exercise: responses equaled those observed prior to bed rest. Sprint speed from a standing start was maintained at pre-bed rest levels when daily LBNP exercise accompanied bed rest (pre-bed rest: 5.5 ± 0.2 meter/sec; post-bed rest: 5.2 ± 0.3). However, bed rest without daily LBNP exercise reduced sprint speed from 5.5 ± 0.2 to 4.6 ± 0.3 meter/sec, or 16% below pre-bed rest control levels ($p < 0.05$). During a walking test on a narrow rail, subjects walked longer following bed rest with daily exercise (12.9 ± 0.9 sec pre-bed rest vs. 11.5 ± 1.9 sec post-bed rest; NS) than after bed rest without exercise (13.5 ± 0.6 pre-bed rest vs. 6.7 ± 1.5 post-bed rest; $p < 0.05$). Calf concentric and eccentric muscle strength, as assessed by peak ankle joint torque, remained at control levels after bed rest with daily LBNP exercise, and isometric strength actually tended to increase with the LBNP exercise treatment ($p = 0.19$). However, apparent reductions in plantarflexor strength after non-exercise bed rest were not statistically significant ($p > 0.07$).

Two weeks of bed rest reduced orthostatic tolerance 24% as assessed by LBNP (time to presyncope), and an essentially identical reduction in tolerance was seen after bed rest with daily LBNP exercise. Supine hematocrit increased from 39.7 ± 1.2 hematocrit units to 42.3 ± 0.9 (2.6 units, $p < 0.05$) after two weeks of non-exercise bed rest, yet no significant increase was seen in hematocrit after bed rest with LBNP exercise. Two weeks of bed rest tended to reduce plasma volume ($p = 0.25$), and LBNP exercise during bed rest appeared to counteract this effect to some extent, although these trends were not statistically significant. Results include data from one subject who exhibited an anomalous increase in plasma and blood volume after two weeks of bed rest without exercise. Exercise during bed rest increased fluid intake on average 413 ml per day (22%) relative to non-exercise bed rest conditions. Five of the seven subjects reported that daily LBNP exercise improved the quality of their sleep during bed rest.

Results from this two-week bed rest study clearly indicate that 40 min per day of supine LBNP exercise at 1.0-1.2 body weights and 40-80% of peak VO_2 maintains upright exercise function and other normally deconditioned variables at pre-bed rest levels. Why did daily LBNP exercise apparently protect orthostatic tolerance in our previous 5-day bed rest study (Lee *et al.*, 1997), yet did not protect tolerance in the two-week study? Use of 5 min of *static* (resting) 50-60 mm Hg LBNP following LBNP exercise in the previous investigation provides one possible explanation for the different findings of the two studies. Static upright posture or LBNP after strenuous exercise probably provides a substantially greater orthostatic stress than does the same stimulus after resting, non-exercise conditions. Therefore, imposition of several minutes of static, resting LBNP after the LBNP exercise session, as was done in our previous 5-day bed rest study, may restore the protective effect of LBNP on orthostatic tolerance which we observed in that prior study, while still keeping the total countermeasure session to less than one hour.

Alternatively, mechanisms of orthostatic intolerance after two weeks of bed rest may be fundamentally different than mechanisms of orthostatic intolerance after five days of bed rest. For example, it is possible that hypovolemia alone explains predominantly post-bed rest orthostatic intolerance in the shorter study, whereas other mechanisms (baroreflex resetting, cerebral vascular acclimation to chronic cerebral hypertension, vestibular acclimation to simulated microgravity) predominate after two weeks of bed rest. Therefore, while daily LBNP exercise largely alleviates hypovolemia seen within a few days of bed rest, the exercise may not by itself adequately counteract all features of longer-term acclimation.

During this LBNP study we also investigated cerebrovascular responses by measuring cerebral blood flow velocity and cerebral tissue oxygenation prior to fainting, during LBNP as an orthostatic stress. During the initial stages of LBNP stress, when blood pressure decreases and heart rate is elevated, these cardiac responses are caused by blood pooling in the legs. But as LBNP continues over time, cardiac insufficiency occurs prior to fainting. We focused on the two minute period prior to the fainting endpoint at which point LBNP was stopped. In a typical subject, blood pressure continued to decrease gradually as fainting was approached. Heart rate decreased rapidly in the final 30 sec, signifying cardiovascular insufficiency just prior to fainting. Cerebral blood flow velocity decreased just prior to fainting. Cerebral tissue oxygenation gradually decreased as fainting was approached.

The new finding from this study is that both cerebral blood flow velocity and cerebral oxygenation decrease prior to fainting. The decrease of cerebral blood flow velocity could be explained as a decrease of cerebral blood flow or a dilatation of the middle cerebral artery. The decrease of cerebral tissue oxygenation could be explained as a decrease of cerebral blood flow or less likely, a decrease of cerebral tissue metabolism. Therefore, it is probable that cerebral blood flow decreases just prior to fainting. In conclusion, during LBNP-induced fainting, cardiac insufficiency primarily occurs, resulting in the failure of cerebrovascular circulation. This study improves the understanding of postflight fainting in astronauts and may aid development of ways to prevent it.

Our finding of the magnitude and mechanism of force production by LBNP has important implications for simulating gravity in space and increasing weightbearing on Earth without the use of a centrifuge. The use of a different air pressure separating the upper and lower body, such as proposed in this project, distributes the net force uniformly over the entire upper surface of the body. This concept thereby avoids the discomfort of localized high pressures typical of bungee cord harness systems. Variations of blood pressures due to inertial loads with normal gait have been documented in humans and other animals and such variations are important for maintenance of normal vascular structure and function in dependent tissues. LBNP simulates gravitational blood pressures in the lower body circulation, and permits the simultaneous additional impact loading of lower body tissues and blood vessels during exercise. On Earth, this concept of loading could be applied to individual limbs for rehabilitation purposes, such as enhancing bone formation after fracture, or to studies of locally-controlled mechanical stress within tissue. LBNP may also supplement the training effect of upright exercise by increasing the footward force and fluid redistribution imposed by gravity. Separately, lower body positive pressure can be used to speed rehabilitation of patients readjusting to upright posture and ambulation. This latter concept has distinct advantages over the use of swimming pools, parallel bars, and other walking assist devices for rehabilitation.

Our results will help determine exercise regimens and exercise devices needed to maintain crew health during long-duration flight as well as improve our understanding of how exercise can be optimized to maintain cardiovascular and musculoskeletal function in people on Earth. Presently, Mir crew members exercise for 2-3 hours per day at about 50% body weight. Our apparatus allows comfortable loading of lower body tissues at one or more body weights. Thus, we expect that the exercise time required for astronauts and Earth-bound people to maintain musculoskeletal strength can be substantially reduced by optimally-increased levels of exercise loads. For example, a recent study of aged subjects found that muscle strength can be regained through an increased level of exercise loads. Thus, our bed rest results will have direct benefits to improve exercise for astronauts in space, and on Earth for bedridden or inactive aged citizens as well as the public at large.

FY97 Publications, Presentations, and Other Accomplishments:

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Noninvasive Intracranial Diameter and Pressure Measurement Using Ultrasound

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Task Description:

NASA and the National Institutes of Health identified intracranial pressure (ICP) as one of the most important parameters to investigate problems of astronauts in space and head trauma of patients on Earth. Current clinical techniques for measuring pressure in the head, however, require surgery to implant a pressure sensor. The primary objectives of the proposed research are to: 1) refine and validate a noninvasive ultrasound technique for monitoring changes in ICP; 2) examine the effects of simulated and actual microgravity on ICP and cerebrovascular hemodynamics; and 3) facilitate transfer of the technology for clinical use. The device, which was originally developed and patented by co-investigators Yost and Cantrell in 1993 and later modified by Hargens and co-workers in 1996, uses an ultrasonic phase comparison method to measure slight changes in cranial diameter which occur with changes in ICP. The proposed research will be conducted over three years. Year one will involve correlation with ICP in cadavera and optimization of the ultrasound measurement technique in healthy humans. Year two will include measurement of cranial diameter and pulsatility in patients with elevated ICP and in healthy individuals during parabolic flight. In the third year, we will apply the noninvasive ultrasound technique to investigate the effects of 7 days of 6° head-down tilt bedrest on ICP and cerebrovascular hemodynamics. We hypothesize that ICP will increase significantly during head-down tilt, decreasing cerebrovascular reactivity and impairing neurologic function. The proposed research will help refine and validate the noninvasive ultrasound device for future space flight investigations and for clinical use. A noninvasive method for monitoring ICP may aid our understanding of the pathophysiology of space adaptation syndrome and post-flight orthostatic intolerance, and may provide a valuable clinical tool for early diagnosis and treatment of patients with elevated ICP.

Measurement of cranial distance (bone-to-bone) in cadavera**Purpose:**

The purpose of this study was to measure changes in intracranial (bone-to-bone) distance in cadavera.

Methods:

Two fresh cadavera with no sign of head trauma were used in this investigation. A ventricular cannula was inserted into the anterior horn of the left lateral ventricle through a burr hole in the frontal bone. ICP was increased by infusing saline into the ventricle through the cannula or decreased by draining cerebrospinal fluid. To measure ICP directly, a fiber-optic, transducer-tipped catheter was inserted into the subdural space through another burr hole. A variable frequency PPLL ultrasound transducer was taped securely to the temporal area of the head above the right ear. Intracranial distance was measured while ICP was increased in 5-10 mmHg increments from the baseline value to a maximum of approximately 40 mmHg.

Results:

Increased intracranial distance was highly correlated with increased ICP ($r = 0.916$)

Significance:

These results suggest that the ultrasound technique is capable of measuring the small changes in intracranial distance related to altered ICP. Magnitudes of cranial expansion observed in the two fresh cadavera were similar to those reported in the literature for cats and support findings of Hiefetz and Weiss in humans. Future studies will utilize a constant frequency PPLL system which improves signal stability and measurement sensitivity.

Elimination of skin thickness from measurements of cranial distance by multiple reflection technique**Purpose:**

The purpose of this study was to develop a method to eliminate skin thickness artifacts from measurements of cranial distance.

Methods:

Intracranial diameter was continuously monitored in five subjects during whole body tilting from 90° head-up tilt to 10° head-down tilt. Each subject underwent two tilting trials which were identical except for the measurement "lock point:" for Trial 1 the variable frequency PPLL device was locked on the first echo and for Trial 2 the device was locked on the second echo. Cranial diameter was calculated as one-half the difference between the two measured path lengths

Results:

Analyzing data using the above equations, intracranial distance increased 0.067 ± 0.030 mm (mean \pm SE) going from 90° HUT to 10° HDT (an increase of ICP equal to 15-17 mmHg). These indirect measurements of bone-to-bone distance changes agree very well with our direct ICP and for cranial expansion results in cadavera for an ICP elevation of 15 mmHg.

Significance:

Despite the fact that data were derived from two separate tilts for each subject, these indirect measurements of bone-to-bone distance agree well with our data for cranial expansion in cadavera and offer support to the validity of the multiple reflection technique to eliminate skin thickness artifacts. Hardware modifications now allow simultaneous tracking of both reflections, thus eliminating the need for two tilting trials (and associated assumptions), while improving accuracy and precision of the technique.

Continuous analysis of fluctuations in cranial diameter**Purpose:**

The purpose of this pilot study was to measure fluctuations in cranial diameter which result from pulsatile changes in arterial blood pressure and ICP.

Methods:

The subject was placed in supine position with the head rotated. A variable frequency ultrasound transducer was

placed on a temporal area and compressed against the table by the weight of the head. Newly-developed circuitry now permits the rapid tracking of skull expansion. This allows the noninvasive measurement technology for the first time to record dynamic ICP responses. Figure 3A shows pulsatile changes in arterial blood pressure (Finapres finger cuff) and intracranial diameter ("integrator output", arbitrary units) averaged over one cardiac cycle. Part of this proposal is to develop calibration techniques which will convert the arbitrary units into actual ICP changes. Data were collected at the sampling rate of 50 Hz and analyzed with fast Fourier transformation.

Results:

Frequencies of intracranial diameter fluctuations (with transducer compression to eliminate cutaneous microvascular artifacts) correlate well with blood pressure fluctuations.

Significance:

These results demonstrate that we can detect pulsatile fluctuations of intracranial distance continuously using the ultrasound technique. Frequency analysis of intracranial distance measurements will provide a valuable means of detecting changes in ICP because (1) characteristics of ICP pulses change predictably with ICP (elevated mean ICP increases amplitude of ICP pulsations), and (2) intracranial distance fluctuations are directly related to ICP pulses. Recent hardware modifications eliminate the need for transducer compression and allow improved signal resolution.

Current PPLL hardware modifications

Our studies to date have used a customized variable frequency PPLL which measures distance from the transducer to the contralateral inner surface of the skull. To do so, the electronics and timing circuits followed the reflected wave, or "lock point," by varying ultrasonic frequency to hold phase constant. Our modified hardware employs a constant frequency PPLL and new timing circuitry to eliminate contributions to the acoustic waveform from extracranial tissue lying between the transducer and skull.

The device is capable of simultaneously tracking two ultrasonic echoes, using either a single transducer (multiple reflection technique) or a dual-frequency probe. The two measurements may be subtracted electronically in real-time. While the multiple reflection technique is currently the preferred embodiment of our device, the dual-frequency probe method is a simple, viable alternative which will also aid in verification and optimization of the hardware.

Significance:

The above-described hardware modifications allow measurement of intracranial diameter unconfounded by changes in skin thickness, arterial pulsations within the cutaneous microvasculature, or slight mechanical instability of the transducer. In addition, the constant frequency PPLL (as opposed to variable frequency) offers increased signal stability, accuracy, and enhanced signal-to-noise ratio capabilities for our applications.

Considerable Earth benefit is derived from our research. Previously ICP has been measured using invasive techniques by inserting a catheter or probe into the intracranial or intraspinal space. Invasive procedures are time and personnel consuming and represent significant risk to patients. Early noninvasive measurements of ICP will help reduce both the mortality and morbidity associated with head trauma, tumors, and cerebrovascular diseases.

FY97 Publications, Presentations, and Other Accomplishments:

Patent Pending, U. S. Patent #: Undetermined Hargens, A.R., Ballard, R.E., Watenpaugh, D.E., Wilson, M., Cantrell, J.H., and Yost, W.T. "Ultrasonic methods for assessment of changes in intracranial pressure through measurement of skull expansion."

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Baroreflex Function in Rats after Simulated Microgravity

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Funding:

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Task Description:

Prolonged exposure of humans to decreased gravitational forces during space flight results in a number of adverse cardiovascular consequences, often referred to as cardiovascular deconditioning. Prominent among these negative cardiovascular effects are orthostatic intolerance and decreased exercise capacity. Rat hindlimb unweighting is an animal model which simulates weightlessness and results in similar cardiovascular consequences. Cardiovascular reflexes, including arterial and cardiopulmonary baroreflexes, are required for normal adjustment to both orthostatic challenges and exercise. Therefore, the orthostatic intolerance and decreased exercise capacity associated with exposure to microgravity may be due to cardiovascular reflex dysfunction. Our studies test the general hypothesis that hindlimb unweighting in rats results in impaired autonomic reflex control of the sympathetic nervous system. Specifically, we hypothesize that the ability to reflexly increase sympathetic nerve activity in response to decreases in arterial pressure or blood volume will be blunted due to hindlimb unweighting. There are 3 specific aims: 1) to evaluate arterial and cardiopulmonary baroreflex control of renal and lumbar sympathetic nerve activity in conscious rats subjected to 14 days of hindlimb unweighting; 2) to examine the interaction between arterial and cardiopulmonary baroreflex control of sympathetic nerve activity in conscious hindlimb unweighted rats; and 3) to evaluate changes in afferent and/or central nervous system mechanisms in baroreflex regulation of the sympathetic nervous system. These experiments will provide information related to potential mechanisms for orthostatic and exercise intolerance due to microgravity.

EFFECTS OF HINDLIMB UNLOADING ON ARTERIAL BAROREFLEX FUNCTION IN THE CONSCIOUS RAT. Studies evaluating the effects of hindlimb unloading (HU) on arterial baroreflex control of renal (RSNA) and lumbar sympathetic nerve activity (LSNA) were completed. This work has been submitted for publication to the *American Journal of Physiology*, and is in revision. Briefly, HU resulted in attenuated ability of the arterial baroreflex to increase both RSNA and LSNA in response to a hypotensive challenge. Baroreflex control of heart rate was unaltered. These data indicate that baroreflex control of the sympathetic nervous system is depressed by simulated microgravity. Similar changes could account in part for orthostatic intolerance observed after space flight in humans.

ROLE OF BARORECEPTOR AFFERENTS AND CENTRAL NERVOUS SYSTEM PROCESSING IN BAROREFLEX EFFECTS OF HINDLIMB UNLOADING.

These studies evaluated whether the attenuation in baroreflex control of sympathetic nerve activity due to hindlimb unloading (HU) is due to altered baroreceptor afferent activity or changes in central nervous system (CNS) processing of afferent input. Rats were subjected to 14 days of HU. They were then anesthetized with sodium pentobarbital and instrumented for measurement of arterial pressure (MAP), aortic depressor nerve activity (ADNA) as a measure of baroreceptor afferent activity, and efferent RSNA. Baroreceptor afferent function was assessed by relating changes in MAP to ADNA, and central processing was evaluated by relating changes in afferent input (ADNA) to efferent sympathetic outflow (RSNA). As shown in our previous studies, HU exhibited an attenuated ability to reflexly increase RSNA in response to decreases in MAP. The relationship between MAP and ADNA was unaltered by HU. In contrast, the slope of the line relating ADNA to RSNA during decreases in MAP was attenuated in HU rats. These data suggest that baroreceptor afferent function is unaltered by HU. However, the diminished baroreflex control of RSNA following HU is due to altered CNS processing of baroreceptor afferent input.

CONSTRUCTOR RESPONSES IN SOLEUS FEED ARTERIES OF HINDLIMB UNLOADED RATS.

Exposure to HU results in reduced blood flow to some skeletal muscles during exercise. This study tested the hypothesis that HU enhances constriction to norepinephrine (NE) in rat soleus feed arteries (SFA). Rats underwent 14 days on normal cage activity (CON) or HU. SFA were isolated and cannulated, and intraluminal pressure was maintained at 90 cm H₂O. Development of spontaneous tone and constrictions to potassium chloride were not altered by HU. However, constrictions to NE were significantly greater in HU rats compared to CON. These data suggest that 14 days of HU specifically enhances NE-induced constrictions in SFA.

ATTENUATED FOS EXPRESSION IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) OF HINDLIMB UNLOADED RATS AFTER HYDRALAZINE TREATMENT.

This study examined Fos-like immunoreactivity following decreases in MAP in control and HU rats. Rats were subjected to 14 days of HU or CON and instrumented for measurement of MAP and infusion of drugs. In conscious rats, MAP was recorded for a 30 min baseline period and for 90 min following intravenous injection of hydralazine (HDZ) to reduce MAP, or isotonic saline vehicle. Rats were then anesthetized, perfused with paraformaldehyde, and brains were removed and processed for Fos immunoreactivity. Resting MAP and MAP following HDZ treatment were similar in both groups of rats. Numbers of Fos-positive nuclei in the RVLM were similar in both groups after saline treatment, and increased in response to decreases in MAP due to HDZ. However, the number of Fos-positive nuclei in the RVLM after HDZ was less in HU rats (7.7) compared to CON (18.3). These data are consistent with previous data indicating that HU results in attenuated baroreflex mediated increases in SNA, and suggest that this alteration may be associated with decreased neural activation in the RVLM.

RESPONSE TO HEMORRHAGE IN HINDLIMB UNLOADED RATS. Previous work indicated that HU in rats results in attenuated baroreflex sympathoexcitation. This study tested the hypothesis that HU rats exhibit reduced sympathetic responses during hemorrhage. Rats were either subjected to HU or CON, and instrumented to record MAP, heart rate (HR) and LSNA. Two days following surgery, MAP was similar in both groups of conscious rats, while HR was increased in HU rats. Rats were subjected to hemorrhage (3 ml/kg/min) until MAP < 40 mmHg. During blood loss, both groups exhibited reflex increases in LSNA. However, the increase in LSNA was attenuated in HU compared to CON rats. The blood loss required to decrease MAP to < 40 mmHg was similar in both groups. Results indicate that the ability to reflexly increase LSNA during hemorrhage is reduced by HU. However, this does not interfere with the ability to maintain MAP. Other compensatory mechanisms may act to maintain MAP during hemorrhage in HU rats despite reduced sympathetic activity.

This work seeks to understand the mechanisms involved in the orthostatic intolerance and reduced exercise capacity observed following space flight. The data suggest that there may be alterations in both the neural control mechanisms and vascular mechanisms required for normal orthostatic tolerance. The arterial baroreflex is less capable of initiating increases in sympathetic nerve activity following a period of simulated microgravity. This appears to be due to a deficit in central nervous system processing of baroreceptor afferent input. Reduced baroreflex mediated increases in activity of the sympathetic nervous system could contribute to orthostatic

intolerance. In addition to these changes in cardiovascular reflex function, there appear to be vascular alterations which could contribute to reduced exercise capacity. Feed arteries supplying the soleus muscle appear to exhibit enhanced constrictor responses to norepinephrine. Enhanced vasoconstriction of skeletal muscle during exercise could contribute to altered distribution of cardiac output during exercise, and thus result in diminished exercise capacity. Understanding of the underlying mechanisms behind orthostatic intolerance and diminished exercise capacity is essential to the development of rational strategies to counteract these potential consequences of prolonged exposure to microgravity.

FY97 Publications, Presentations, and Other Accomplishments:

Jasperse, J.L., Hasser, E.M., Friskey, S.A., Smith, J.A., and Laughlin, M.H. "Constrictor responses in soleus feed arteries of hindlimb unweighted rats." *FASEB J.* (1997).

Moffitt, J.A., Foley, C.M., Schadt, J.C., Friskey, J.A., and Hasser, E.M. "Effects of hindlimb unweighting on baroreceptor afferent signaling." *FASEB J.* (1997).

Mechanisms of Heterogeneity in the Lung

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Funding:

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Students Funded Under Research: 0 Post-Doctoral Associates: 0
FY 1997 Funding: \$ 118,987
Joint Agency Participation: NIH/National Heart, Lung, and Blood Institute

Task Description:

While the original studies of regional pulmonary blood flow distribution suggested that nearly all of the heterogeneity was explained on the basis of a gravitational gradient, recent flow studies utilizing high-resolution techniques in both prone and supine postures demonstrated a far greater extent of flow heterogeneity, with only about 4% of the total heterogeneity attributed to the influence of gravity. However, as the latter studies were conducted at 1.0 G, the posture changes were necessarily associated with mediastinal shifts, which in themselves could alter the distribution of blood flow by different forces applied to the pulmonary arteries and veins. This project will investigate the distribution of pulmonary blood flow in anesthetized pigs in both prone and supine postures during the 0.0, 1.0, and 2.0 G conditions attained during parabolic profile flights on the KC-135. The distribution of blood flow will be measured by intravenous injection of different 15 μ m fluorescent microspheres (FMS) during each position and gravitational state, along with magnetic measurements of mediastinal shift. After the lungs are dried and cut, maps of blood flow distribution based on intensity of the different fluorescent markers will be constructed. Flow distributions at the different conditions will be compared by ANOVA to test the hypothesis that exposure to microgravity produces a small redistribution of blood flow in comparison to the prone posture at 1.0 G, but that microgravity causes a larger redistribution of flow in comparison to the supine posture at 1.0 G. These studies will provide information on the importance of mediastinal shift as a determinant of blood flow distribution in the lung, and provide new information on the mechanism for hypoxia observed in some humans in the supine posture.

*State Dependent Aspects of Cognition***Principal Investigator:**

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Solicitation: US

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Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

Joint Agency Participation: NIMH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Mental activity occurs not only during waking, but during REM sleep and non-REM (NREM) sleep as well. The formal cognitive characteristics of these three states are distinctly different from one. For example, the stimulus source of perception shifts from predominantly external in waking to predominantly internal in sleep. Simultaneously, the memory of such processing falls dramatically. It is our goal to identify and characterize aspects of cognitive processes that are dependent on the state of the brain. Our specific goals are:

- I. To study changes in cognitive processes across sleep-wake stages in a sleep laboratory setting
 - A. Study the effects of sleep-wake state on cognitive functions by performing standard behavioral tests before sleep and following arousals from different stages of sleep
 1. Characterize the effects of sleep-wake state on the ability to re-orient attention by quantifying reaction speeds on the perceptual cuing test
 2. Characterize the effects of sleep-wake state on the ability to access associational memory nets by quantifying reaction speeds and accuracy on the semantic priming test
 - B. Systematize self-report techniques for studying internal aspects of cognition that change across sleep-wake states
 1. Characterize orientational instability in a new sleep mentation data set obtained from identified physiological states
 2. Measure personal, spatial, and temporal discontinuities in this data set using various techniques including mathematical graph representations and a novel "spliced dream" technique
- II. To develop and test methods of studying state-dependent cognition in home settings
 - A. Further confirm the efficacy of the Nightcap home recording system in determining sleep states by comparing results obtained from this system with those obtained simultaneously using a standard polysomnograph system

B. Compare sleep cycles as measured in a home setting with data obtained from the same subjects in the laboratory

C. Compare results of behavioral tests and self-reports collected in a home setting with those obtained from the same subjects in the laboratory

During the last year, we have explored the relationship (1) between sleep and learning and (2) between sleep and associative memory processes.

In the first set of experiments, we found that learning can be strongly dependent on sleep quality. Procedural learning measured in a visual discrimination task resulted in long-term improvement after a single training session. When tested within 24 h of training, learning required at least 6 h of post-training sleep and was proportional to the amount of sleep in excess of 6 h. For subjects averaging 8 h of sleep, improvement was proportional to the amount of slow wave sleep in the first quarter of the night (SWS_1) as well as the amount of REM sleep in the last quarter (REM_4). A two-step process, modeling throughput as the product $SWS_1 \times REM_4$, accounted for 80% of inter-subject variance.

In the second set of experiments, we found that when subjects were awakened from REM sleep, they accessed different associations than when awakened from non-REM sleep. Semantic priming can be used to quantify the strength of associative links between pairs of words; it is thought to measure the automatic spread of activation from a "node" representing one word to nodes representing semantically related words. Semantic priming can thus be used to test for global alterations in the strengths of associative links across the wake-sleep cycle. Contrary to the normal pattern of priming, subjects awakened from REM sleep showed greater priming by weak primes than by strong primes ($p = 0.01$). This result was seen in each of three protocols ($p = 0.10 - 0.15$ for each). In contrast, strong priming exceeded weak priming in NREM sleep. The shift in weak priming seen after awakenings from REM sleep may reflect a shift in associative memory systems which has been hypothesized to underlie the bizarre and hyper-associative character of REM-sleep dreaming. These findings indicate that cognition during REM sleep is qualitatively different from that of waking. The results seen here may be explained in terms of the known changes in brainstem activity which control the shift into and maintenance of REM sleep.

Sleep plays a still poorly defined role in modulating the health of individuals. One aspect of this role is believed to be the maintenance of cognitive systems, including memory systems. The studies here show that well-formed sleep, with deep sleep at the start of the night and REM sleep at the end of the night, might be necessary for the efficient learning of at least some tasks. Understanding the nature of this relationship and its possible origin could lead to improved learning, especially of manual and visual tasks. This would be of obvious importance both in space and on Earth. Since space flight can lead to a general deterioration of sleep quality, learning of new tasks within the environment of space may be diminished unless proper care is taken to ensure good sleep. This would be equally true on Earth. Of particular interest to most people is the possible relationship of deep, "slow-wave" sleep to learning. Since the amount of this sleep diminishes as one ages, it is possible that the age-related decrease in learning capacity may be due in part to this deterioration of sleep. Drug interventions are known which can restore this slow-wave sleep, but it is not known whether they can also reverse a portion of the age-dependent loss in memory and learning.

FY97 Publications, Presentations, and Other Accomplishments:

Hobson, J.A. "Dreaming" in "Encyclopedia of Neuroscience." Edited by: Adelman, G. 2nd Edition (In Press).

Hobson, J.A. "Sleep" in "A Busy Night." Scientific American Books (In Press).

Hobson, J.A. "Sleep (behavioral and cellular mechanisms)" in "Encyclopedia of Neuroscience." Edited by: Adelman, G. 2nd Edition (In Press).

Hobson, J.A. "Sleep, functional theories" in "Encyclopedia of Neuroscience." Edited by: Adelman, G. 2nd Edition (In Press).

Hobson, J.A. "A review of trauma and dreams." *Nature Med.*, 3, 243 (1996).

Hobson, J.A. and Silvestri, R. "The restless brain: Disorders of sleep and dreaming." *Odyssey*, 2, 24-31 (1996).

Hobson, J.A., Stickgold, R., and Pace-Schott, E.F. "The neuropsychology of REM sleep dreaming." *A NeuroReport Review* (In Press).

Kahn, D., Pace-Schott, E., and Hobson, J.A. "Consciousness in waking and dreaming: The roles of neuronal oscillation and neuromodulation in determining similarities and differences." *Neuroscience*, 13-38 (1997).

Leslie, K.R., Stickgold, R.A., DiZio, P., Lackner, J., and Hobson, J.A. "Sleep and vestibular adaptation: Implications for function in microgravity." *J. Vestib. Res.* (In Press).

Leslie, K.R., Stickgold, R.A., DiZio, P., Lackner, J., and Hobson, J.A. "The effect of optokinetic stimulation on daytime sleepiness." *Archives Italiennes de Biologie*, 135(3), 219-228 (1997).

Nordby, H., Hugdahl, K., Stickgold, R., Bronnick, K.S., and Hobson, J.A. "Event-related potentials (ERPs) to deviant auditory stimuli during sleep and waking." *NeuroReport*, 7, 1082-1086 (1996).

Pace-Schott, E.F., Stickgold, R., and Hobson, J.A. "Memory processes within dreaming: Methodological issues." *Sleep Res.*, 26, 277 (1996).

Stickgold, R., Sangodeyi, F., and Hobson, J.A. "Judges cannot identify thematic coherence in dream reports with discontinuities." *Sleep Res.*, 26, 278 (1997).

Sutton, J.P., Breiter, H.C., Caplan, J.B., Huang-Hellinger, P.R., Kwong, K.K., Hobson, J.A., and Rosen, B.R. "Human brain activation during REM sleep detected by fMRI." *Soc. Neurosci.*, 22, 273 (1996).

Vitamin D RDA from Supplement of Light

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$197,508

Task Description:

Although it has been generally recommended that the RDA for young and middle age adults for vitamin D is 200 IU (5 micrograms), this is only adequate as long as they are exposed to sunlight. This issue is of particular interest to NASA as they begin to develop plans for longer duration spaceflights for the space station program. The goal of this research program is to critically investigate the RDA for vitamin D for healthy, young and middle-aged male and female adults. We also plan to investigate whether passive exposure to simulated sunlight can produce enough vitamin D in the skin to satisfy the body's vitamin D needs. This will be accomplished in two ways: 1) Establishing the vitamin D daily requirement for healthy young and middle-aged male and female adults by supplementing groups of adults with a vitamin D supplement of either 0, 200, 400, 600, or 800 IU of vitamin D₂ each day beginning in October and ending in March. During this time, the cutaneous synthesis of vitamin D is inadequate in the Boston area. The determination of circulating concentrations of vitamin D, 25-hydroxyvitamin D, parathyroid hormone and other parameters of calcium metabolism will be evaluated throughout the study; and 2) Determining the effect of controlled exposure to simulated sunlight 3 times a week on circulating concentrations of vitamin D, 25-hydroxyvitamin D, parathyroid hormone and other parameters of calcium metabolism between October and March. Study results will provide NASA with information that will be valuable in determining the amount of vitamin D that astronauts should take during long-duration space flights for the space station program. In addition, this study will provide information on the possible use of simulated sunlight on space station as a means of ensuring that astronauts will produce enough vitamin D to satisfy their bodies' needs. The ultimate outcome of this study should provide NASA with important information about how much vitamin D is necessary in the absence of sunlight to prevent vitamin D insufficiency and vitamin D deficiency during long-duration space flight.

We have recruited young and middle-aged adults and exposed them to simulated solar ultraviolet B radiation three times a week for a period of 7 weeks. Our initial evaluation was to measure the circulating concentrations of vitamin D levels in a subset of these volunteers who had their arms, face, and legs exposed to this simulated sunlight. The circulating concentrations of vitamin D increased from 1.1 ± 0.3 (means + SM) to 5.4 ± 0.4 ng/ml. Once we were able to demonstrate that this simulated sunlight source enhanced the production of vitamin D in the skin which translated into increased circulating concentrations of vitamin D in the volunteers, we then measured circulating concentrations of 25(OH)D over a period of 7 weeks. After the first week, there was a $46 \pm 3\%$ increase in circulating concentrations of 25(OH)D in the irradiated subjects. The change in circulating 25(OH)D gradually increased and peaked at 160% by week 5 that persisted at week 7. These data

clearly demonstrate that exposure of healthy young and middle-aged adults to simulated solar ultraviolet B radiation can enhance the production of cutaneous vitamin D synthesis which is translated into marked increases in circulating concentrations of 25(OH)D. These data provide a sound basis for considering placing an ultraviolet B light source on space flights that are for long-duration space travel.

The results from this ongoing research program has direct application for human health and disease on Earth. The results show that exposure to an artificial ultraviolet B light source not only produces vitamin D in the skin that enhances the blood levels of vitamin D, but also increases the major circulating form of vitamin D, 1,25-dihydroxyvitamin D. It is 25-dihydroxyvitamin D that acts a substrate for the production of its active form 1,25-dihydroxyvitamin D. There is mounting evidence that vitamin D deficiency is a major health problem for adults over the age of 50 years. This is especially true for adults over 70 years who are institutionalized or are in nursing home settings. The development of indoor lighting that contains a small amount of ultraviolet B radiation could be of great benefit by passively providing those exposed to this light source their daily requirement for vitamin D. Since vitamin D deficiency exacerbates osteoporosis, increases risk of fracture, and causes the bone disease osteomalacia, this simple method could have a significant health impact for bone health of the elderly.

FY97 Publications, Presentations, and Other Accomplishments:

Holick, M.F. "Photobiology of vitamin D" in "Vitamin D." Edited by: Feldman, D. Academic Press: San Diego (In Press).

Holick, M.F. "Reasonable vitamin D daily allowance" in "Serono Symposia Series, Nutritional Aspects of Osteoporosis (May 1997)." Edited by: Burckhardt, P. Springer Verlag (In Press).

Holick, M.F. "A perspective on the impact of weightlessness on calcium and bone metabolism." International Workshop on Bone Research In Space (Japan). Bone (In Press).

Dietary Oxalate and Stone Risk

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$82,808

Task Description:

During extended space flight, the formation of a calcium oxalate kidney stone becomes a real risk in otherwise healthy individuals. Of the male Caucasian astronauts, those most susceptible to oxalate stone disease, approximately 25% will be genetically predisposed to the disease. As stone risk increases with microgravity a "stone event" during extended space flight is highly likely unless appropriate countermeasures are adopted. Recent research in our laboratory indicates that dietary oxalate makes a major contribution to urinary oxalate excretion in most individuals. However, the relationship between the amount of oxalate in the diet and the influence of modifiers of oxalate absorption on oxalate excretion and stone risk remain unknown. The long term objective of the research in this proposal is to determine whether modification of the amount of oxalate absorbed from the diet is an effective approach for decreasing oxalate stone disease in susceptible individuals. Utilizing a new technique we have developed for the analysis of the oxalate in foods, the level of dietary oxalate will be controlled in three diets to be consumed by 12 healthy individuals. The current diet consumed by space shuttle crew members contains numerous oxalate-rich items. To make this proposal relevant to the Space Program the effects of controlled diets of high and low oxalate content selected from this menu will be compared. Other factors that may influence oxalate absorption and stone risk analytes in urine will also be controlled. To identify the maximum changes possible with this dietary modification the effects of an ultra-low oxalate controlled diet (< 6 mg/day) will be examined. To simulate space flight, urinary excretions will also be examined on an uncontrolled, self-selected space diet modified to lower oxalate intake. This research will clarify whether a reduction in dietary oxalate is a practical measure to reduce stone risk.

Methods for the accurate and reproducible estimation of the oxalate content of foods are being evaluated. Two direct methods are being compared, capillary electrophoresis and ion chromatography. Ion chromatography shows the most overall reliability, with a greater sensitivity and accuracy than capillary electrophoresis. The main advantage of capillary electrophoresis over ion chromatography is its rapidity. In both procedures, the binding of food components to the analytical columns reduces their resolving capabilities and alters analysis times. Ultimately column replacement is required.

Factors critical for the complete dissolution of calcium oxalate in foods have been assessed. Complete dissolution, as judged by maximal extraction, was obtained with 0.2 M acid, either HCl or H₃PO₄, and an

extraction temperature of 60°C. Whether a significant fraction of the oxalate in plants exists in a soluble rather than crystalline form cannot be determined in such experiments despite reports to the contrary. Practically all of the oxalate in spinach, wheat bran, and apple can be solubilized by infinite dilution of homogenates in water. Because of the ubiquity of Ca^{2+} in biological systems including plants, we consider it most likely that the bulk of plant oxalate will exist as crystalline calcium oxalate and not as the free anion.

The oxalate content of foods is being compiled to prepare menus of known oxalate content. Individuals will soon be recruited to test the effects of diets of varied oxalate content on urinary oxalate excretion.

This project is related to kidney stone disease. The risk of forming stones is increased in space due to bone dissolution, but it is still a substantial risk on Earth. 10 - 15% of Caucasian males are expected to form at least a single kidney stone during their lifetime. This research should help clarify the contribution of dietary oxalate to urinary oxalate excretion and will provide a compilation of values of the oxalate contents of foods. It should be possible to develop dietary guidelines that will reduce the contribution of dietary oxalate to stone risk both in space and on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Holmes, R.P. "Dietary oxalate and stone disease." ROCK Society Meeting, Steamboat Springs (July, 1997).

Holmes, R.P., Assimos, D.G., and Goodman, H.O. "The contribution of dietary oxalate to urinary oxalate excretion." Special Session on Stone Disease, 1997 AUA annual Meeting, New Orleans (April, 1997).

Monitoring Physiological Variables with Membrane Probes

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 0

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Task Description:

Microdialysis and ultrafiltration are two techniques based on membrane probes. These probes can be used for continuous *in vivo* measurements of low molecular weight substances. Membrane probes were developed to study some specific electrolytes (sodium, potassium, and chloride), and metabolites (glucose and lactate) which are affected by the microgravity environment of space flight. The relationship between the concentrations of these substances in blood and subcutaneous probe samples were determined to validate the use of subcutaneous samples in place of blood samples in physiological studies. The rat hind limb suspension model was used to simulate microgravity and demonstrate the use of these probes for microgravity studies of electrolyte changes due to fluid shifts and changes. Intramuscular probes were developed to study the effects of hind limb elevation on changes in glucose and lactate within the muscle. On-line monitoring systems were developed for the metabolites. Enzyme electrodes were adapted for monitoring of glucose and lactate and were incorporated into a low dead volume flow cell. Ion selective electrodes for electrolytes were tested for possible use in on-line sensors.

The rat head-down tilt model of microgravity was used to study changes in interstitial fluid concentrations of the electrolytes sodium, potassium, and chloride and the metabolites glucose and lactate. Preliminary data were presented in last year's task book. Data collection and analysis were completed. Ultrafiltration and microdialysis membrane probes were implanted subcutaneously in rats. They were monitored for 3 to 7 days under baseline conditions, suspended in a head-down tilt position for 14 days and allowed to recover for 7 days. Some rats were unable to adapt to the head-down tilt position and suspension was ended earlier than 14 days.

Six rats were used for statistical analysis of analyte changes during head-down tilt and recovery. Table 1 shows the concentrations of sodium in probe and plasma samples under different conditions.

Table 1. Average subcutaneous interstitial fluid and plasma sodium ion concentrations (meq/L) during baseline, head-down tilt and recovery periods.

	<u>Ultrafiltration</u>	<u>Microdialysis</u>	<u>Plasma</u>
Baseline	135 ± 4	137 ± 7	138 ± 5
Head-down	137 ± 7	138 ± 5	142 ± 7
Recovery	134 ± 4	133 ± 5	137 ± 6

Average sodium concentrations were significantly higher in both ultrafiltration ($p = 0.006$) and microdialysis probe ($p = 0.04$) samples and in plasma ($p = 0.08$) samples in the head-down tilt position than under baseline conditions. With return to normal position during the recovery phase the average sodium concentration decreased in all sample types. For ultrafiltration, the average sodium concentration of the recovery samples was significantly lower than head-down tilt ($p = 0.0006$) and also slightly lower than the baseline level ($p = 0.05$). For microdialysis samples, the average recovery concentration was also significantly lower than the head-down ($p < 0.001$) and baseline ($p < 0.001$) concentrations.

Average plasma sodium levels were higher than both ultrafiltrate and microdialysate concentrations. The differences between ultrafiltrate and plasma were significant in the baseline ($p = 0.02$) and head-down tilt positions ($p = 0.05$). The difference between microdialysate and plasma were significant only in the head-down ($p = 0.02$) position.

Responses of electrolyte concentrations of individual animals to the head-down tilt varied in pattern and magnitude. For example, all rats showed an elevation in sodium at some time during the head-down tilt; two of the rats showed an immediate increase within 24 hours, two of the rats showed a delayed sodium elevation, and the other two showed an initial decline in interstitial sodium followed by an elevation above baseline.

Average potassium concentrations during baseline, head-down tilt, and recovery periods are shown in Table 2.

Table 2. Average subcutaneous interstitial fluid and plasma potassium concentrations (meq/L) during baseline, head-down tilt, and recovery periods.

	<u>Ultrafiltration</u>	<u>Microdialysis</u>	<u>Plasma</u>
Baseline	4.0 ± 0.5	3.9 ± 0.8	3.7 ± 0.3
Head Down	3.9 ± 0.6	3.9 ± 0.4	3.9 ± 0.1
Recovery	4.5 ± 0.4	3.9 ± 0.3	4.7 ± 0.4

The average potassium was not significantly different between baseline and head-down suspension in either probe samples or plasma samples. Average potassium during recovery was higher than head-down suspension in ultrafiltrate ($p = 0.0001$) and plasma ($p = 0.002$) samples, but not significantly different from baseline. No significant differences were seen in microdialysis.

Differences in individual response were observed for potassium. Five of the six rats showed a decrease in interstitial potassium with head-down tilt. Two of these rats showed an immediate but brief increase in potassium before the decline. In three of these rats, the potassium started to rise again during the head-down tilt. In another rat, the decline continued two days into the recovery period before beginning to rise. All rats returned to baseline concentrations during recovery, but one rat rose above baseline for two days before coming back down to baseline.

Average chloride concentrations are shown in Table 3. During head-down tilt, average chloride concentrations increased significantly above baseline in ultrafiltrate ($p = 0.004$) and microdialysate ($p = 0.002$) samples. There was considerable individual variability in time after initiation of head-down tilt and duration of the increase. With return to baseline position, the chloride decreased in ultrafiltrate and plasma samples. Recovery concentrations are not significantly different than baseline concentrations.

Chloride concentrations in microdialysate samples are higher than ultrafiltrate concentrations. This would indicate that microdialysate *in vivo* recovery is less than 100%. The Ringer's perfusion fluid in these studies has as chloride concentration of 156 meq/L. Therefore, a lower recovery results in a higher chloride concentration.

Table 3. Average subcutaneous interstitial fluid and plasma chloride ion concentrations (meq/L) during baseline, head-down tilt and recovery periods.

	<u>Ultrafiltrate</u>	<u>Microdialysate</u>	<u>Plasma</u>
Baseline	104 ± 7	109 ± 5	106 ± 9
Head-down	108 ± 8	111 ± 9	112 ± 7
Recovery	105 ± 7	111 ± 7	102 ± 8

Average lactate concentration was elevated above baseline during head-down tilt in both probe and plasma samples (Table 4). During the recovery period the lactate concentrations declined to baseline levels. The difference between baseline and head-down tilt concentrations was significant only in ultrafiltrate samples ($p < 0.0001$). The difference between head-down tilt and recovery concentrations was significant in both ultrafiltrates ($p < 0.0001$) and microdialysates ($p = 0.01$). One difference between lactate and the electrolytes is the magnitude of the difference between subcutaneous and plasma concentrations. Both ultrafiltrate ($p = 0.0002$) and microdialysate ($p = 0.0005$) concentrations were significantly higher than plasma concentrations.

In contrast to the other analytes, which remained relatively stable during the baseline periods, the lactate concentration rose gradually indicating that the presence of the probes is causing lactate formation within the tissue.

Table 4. Average subcutaneous interstitial fluid and plasma lactate concentrations (mM) during baseline, head-down tilt and recovery periods.

	<u>Ultrafiltrate</u>	<u>Microdialysate</u>	<u>Plasma</u>
Baseline	4.4 ± 2.5	3.8 ± 1.6	1.5 ± 0.5
Head-down	6.9 ± 2.9	4.1 ± 2.4	2.4 ± 1.6
Recovery	4.5 ± 2.0	3.4 ± 1.3	1.0 ± .21

Average glucose concentrations during baseline, head-down tilt and recovery periods are shown in Table 5. The ultrafiltration glucose data follows the same trends as lactate, sodium, and chloride, with an elevation above baseline during head-down tilt and a return to baseline concentrations with return to the normal position. However, none of the differences was significant.

Table 5. Average subcutaneous interstitial fluid and plasma glucose concentrations (mM).

	<u>Ultrafiltration</u>	<u>Microdialysis</u>	<u>Plasma</u>
Baseline	5.2 ± 1.3	4.8 ± 0.3	4.5 ± 0.4
Head-Down	6.0 ± 2.4	6	
Recovery	4.0 ± 0.0		

For the continuous monitoring of glucose or lactate, thin-layer cross-flow amperometric detectors with enzyme modified working electrodes were developed. The enzymes glucose oxidase (GOX) or lactate oxidase (LOX) were covalently immobilized in an osmium redox polymer film on the electrode surface. The electrodes were then over-coated with another membrane to limit transport of interferents and improve linearity. Two types of over-coating membranes were tested: (1) cellulose acetate and Nafion and (2) a polycarbonate membrane. The polycarbonate membrane resulted in better reproducibility better selectivity against interferents.

The membrane sampling probes developed and tested are proving to be effective for continuous monitoring of a number of analytes which are routinely monitored in hospital situations for many different human diseases and conditions. The most significant advantage of these probes is that they allow monitoring without removal of blood. They also remove the need for repeated punctures and/or vascular access. Premature infants are monitored frequently for electrolytes and glucose. Because of their small size the withdrawal of blood for monitoring creates medical problems, and these infants must often receive blood transfusions to replace blood taken for monitoring. Therefore, physicians must constantly weigh the benefits of close monitoring with the disadvantages of transfusion. We have already demonstrated that we can continually monitor rats weighing 300 g for these analytes. This method of monitoring does not result in blood loss, so infants can be monitored as frequently as necessary to insure good metabolic control. An additional advantage to this method is that it does not require vascular access which is difficult to obtain in these small patients. Because of the difficulty in obtaining blood from a vein, samples are frequently obtained by puncturing the heel of the infant. This is a painful procedure. Since it is carried out frequently, the procedure disturbs the sleep of these patients and might possibly lead to some future psychological problems. Use of probes to monitor these infants would make sampling easier for the staff and less painful for the patient. Continuous monitoring could prevent such problems as brain damage resulting from hypoglycemia. Burn patients are another group of patients who would benefit from monitoring by probes. These patients are also very unstable and require frequent monitoring.

Since the membranes of these probes allow only low molecular weight substances to pass into the sample they are free of pathogens. Use of these probes in individuals with blood-borne diseases, who need frequent samples taken, could decrease the risks to staff assigned to obtain and analyze the samples.

The use of probes and sensors for continuous monitoring of glucose could be one of the most useful outcomes of this research for human medicine. Diabetes is the major disease with glucose abnormalities. Glucose monitoring is a necessity for maintenance of good health and for the reduction of morbidity and mortality among diabetics. Most diabetics do insufficient monitoring because of the pain and inconvenience involved in repeated blood sampling and testing. A painless, continuous monitoring system which could be developed as an extension of this research would decrease the morbidity and mortality among diabetics. Also, this would significantly reduce the \$14 billion annual national medical cost of diabetes.

In addition, this monitoring system would greatly facilitate diabetes research using small animal models. Monitoring glucose in these animals is now limited by the volume of blood which can be obtained.

The one analyte that we have found to be different in subcutaneous tissue and plasma is lactate. For this analyte the probe will not be an effective substitute for blood. However, the probes do offer a new technique for studying the metabolic pathways involving lactate in skin and subcutaneous tissue. Previously there was no method of measuring differences in concentration of analytes *in vivo* different tissues. Therefore, membrane probes offer a method for studying metabolism in different tissues and obtaining better understanding of physiological and pathological processes.

FY97 Publications, Presentations, and Other Accomplishments:

Janle, E.M., Yang, L., and Kissinger, P.T. "Monitoring physiological variables with membrane probes." 12th Man in Space Symposium, Washington, DC (June 8 - 13, 1997).

Neural Control Mechanisms and Body Fluid Homeostasis

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Students Funded Under Research: 5
FY 1997 Funding: \$149,763

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Post-Doctoral Associates: 2

Task Description:

Reduced extracellular fluid volume (hypovolemia) is a common effect of space flight and microgravity. Cardiovascular deconditioning and orthostatic intolerance have been proposed to be consequences of hypovolemia. Reducing hypovolemia or its consequences under conditions of microgravity will require an increased understanding about the mechanisms which maintain body fluid homeostasis.

Body fluid balance depends on reflexes to control renal function and on ingestive behaviors (e.g., drinking, thirst). Although renal mechanisms can slow the rate of fluid loss, drinking is necessary for an ultimate restoration of homeostasis. The maintenance of extracellular volume requires that the central nervous system receives and processes information about the status of body water and sodium. Several types of receptors located through the body normally provide this afferent input. However, under severe environmental challenge or in pathological states, the input and processing of information from receptor systems may be distorted and disrupted. At the present time, there is only limited understanding about the nature of interactions of sensory systems that signal the status of body fluids. There is even less known about how the brain processes this information that is critical for maintaining fluid homeostasis and cardiovascular fitness.

The present proposal builds upon this laboratory's prior investigations of fluid-related afferent signaling and central processing. The proposed research will employ a recently developed model in the rat that permits the investigation of interactive hormonal (angiotensin) and neural (arterial blood pressure) afferent signals that control hypovolemic thirst. Experiments using this model will generate important new information about basic physiological mechanisms that maintain and restore body fluid homeostasis. An increased understanding of these neurobiological processes will contribute to the development of effective countermeasures to microgravity-induced hypovolemia. Such new knowledge will also have relevance for the treatment and well-being of normal individuals exposed to physiological (exercise) and environmental (heat) challenges and of certain types of patients with pathological conditions related to fluid balance (hypertension, congestive heart failure).

The goal of the proposed research is to study the mechanisms of afferent signaling to the brain of information about the status of body fluid balance and to investigate the central neural mechanisms that process this information for the activation of behaviors which restore body fluid homeostasis. That is, in the face of loss of fluids from intracellular or extracellular fluid compartments, animals seek and ingest water and ionic solutions

(particularly Na⁺ solutions) to restore the intracellular and extracellular spaces. Over recent years, our laboratory has generated a substantial body of information indicating that 1) a fall in systemic arterial pressure facilitates the ingestion of rehydrating solutions, and 2) that the actions of brain mono-amine systems (e.g., norepinephrine, serotonin) are critical for precise correction of fluid losses. Because both acute and chronic dehydration are associated with physiological stresses, such as exercise and sustained exposure to microgravity, the present research will aid in achieving a better understanding of how vital information is handled by the nervous system for maintenance of the body's fluid matrix which is critical for health and well-being.

Brain mono-amines exert their actions at several central nervous system nuclei involved in the control of body fluid balance. Many of these nuclei are also innervated by cells that contain glutamate, an excitatory amino acid. Glutamate has been implicated in neural plasticity and may be related to resetting of volume and baroreceptor reflex mechanisms. In turn, resetting of such reflexes may contribute to orthostatic hypotension associated with weightlessness.

Very recently, we have begun to examine the effect of manipulation of excitatory amino acid systems in the brain. First, we investigated the effects of the N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, blood pressure responses and *c-fos* induction (i.e., FOS-immunoreactivity, FOS-ir) in response to intracerebroventricular (icv) angiotensin II (ANG II) injections. MK-801 was administered icv after ANG II injections.

Icv MK-801 (20 nmol for conscious rats, and 60 nmol for anesthetized rats) did not affect mean blood pressure and heart rate, but significantly suppressed icv ANG II-induced pressor responses in both conscious and anesthetized rats. This suggests an involvement of glutamatergic mechanisms in mediating for the effects of icv ANG II. FOS-immunoreactivity after icv ANG II was significantly increased in the subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), median preoptic nuclei (MnPO), supraoptic nuclei (SON), and magnocellular paraventricular nuclei (PVN). Pretreatment with icv MK-801 had no effects on FOS responses in the SFO, OVLT and magnocellular PVN, but quantitatively decreased FOS in the MnPO, SON and the magnocellular PVN. This suggests the central glutamate mechanisms in the forebrain are related to ANG II-induced pressor effects.

In a second series of studies, we examined the role of glutamate in the lateral parabrachial nucleus (LPBN) which has been demonstrated to have a serotonin-related inhibitory role in the control of water and salt intake induced by extracellular fluid depletion. This study examined the role of the glutamate mechanisms in the LPBN in renin-angiotensin mediated water and sodium intake. The non-NMDA (n-NMDA) receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 25 nmol/ μ l) was dissolved in DMSO as a vehicle. Rats were injected with either DNQX or vehicle bilaterally (200 μ l each site) into the LPBN 1 hr after combined treatment with furosemide (FURO; 10 mg/kg, sc) and captopril (CAP; 5 mg/100 g, sc) which induces hypovolemia and hypotension and in turn, both water and 2% NaCl solution intake. Rats treated with DNQX showed significantly enhanced salt and water intake compared with vehicle-treated controls. Significant enhancement of water and salt was also observed at lower doses of DNQX (10 nmol/ μ l and 5 nmol/ μ l). When α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA; 2 nmol/ μ l), a n-NMDA receptor agonist was injected before DNQX, the enhancement in water and salt intake induced by the FURO/CAP treatment was blocked. The results suggest that a glutamate related n-NMDA receptor mechanism is involved in the control of salt and water intake associated with the LPBN.

Humans who have lost sodium and water as a result of exercise and/or high temperature do not drink sufficient amounts of water to replete extracellular fluid volume. This impairment in thirst mechanisms has classically been referred to as voluntary dehydration. Water intake appears to be actively inhibited, and unless appropriate amounts of sodium are provided, drinking will not resume. Dehydration in the heat reduces the body's capacity for evaporative cooling and hence increases the risk of heat stroke. At present, the mechanisms causing voluntary dehydration are unknown. Similar mechanisms causing disordered regulation in microgravity may be activated during exercise. A more complete understanding of the neurobiological control of body fluid homeostasis has relevance to the well-being of healthy individuals under relatively "normal" conditions.

Alterations in body fluid volume have been implicated in several types of cardiovascular pathology. Notable is the work of Guyton and his colleagues and others who have repeatedly demonstrated that expansion of extracellular fluid/blood volume is an antecedent of many forms of hypertension. On the grounds of many experimental analyses, it has been hypothesized that a major trigger for the onset of human essential hypertension is a mismatch of water and salt ingestion in relation to renal excretion. A thorough understanding of the behavioral and reflex mechanisms that determine blood volume is likely to increase our knowledge about the basis of hypertension and related cardiovascular diseases.

FY97 Publications, Presentations, and Other Accomplishments:

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Vestibular Influences on Autonomic Cardiovascular Control

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Funding:

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Solicitation: 96-OLMSA-01
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Post-Doctoral Associates: 0

Task Description:

Although there is substantial evidence that the vestibular and autonomic systems interact, the functional importance of the vestibular input in cardiovascular control is unknown. The goal of this proposal is to determine if activation of vestibular afferents triggers reflexes that engage autonomic pathways involved in cardiovascular regulation, particularly those that will be studied in the Neurolab Project. Our hypothesis is that linear acceleration along the long axis of the body (Z axis), or along the naso-occipital axis (X axis), signaling changes in body orientation with regard to gravity, causes vestibular-induced alterations in autonomic outflow to stabilize blood pressure. Novel scientific approaches include the use of paradigms that will allow us to distinguish between stimulation of semicircular canals and otolith organs. In Specific Aim I, we will use "radial" centrifugation to produce acceleration along the Z axis, and rotation about axes tilted from the vertical (off-vertical axis rotation, OVAR) to produce sinusoidally varying naso-occipital acceleration while monitoring autonomic function. On-center rotation about a vertical axis and "tangential" centrifugation will provide control conditions in which the semicircular canals and otolith organs will be activated. In Specific Aim II, we will use microneurography to determine the effect of vestibular stimulation on sympathetic outflow in normal subjects and in patients with baroreflex failure. In Specific Aim III, we will assess the impact of vestibular reflexes on orthostatic tolerance. In Specific Aim IV, we will explore the effect of promethazine on vestibular and autonomic reflexes and reflex interactions.

In summary, this proposal will use state-of-the-art techniques to determine in a definitive way whether vestibular reflexes have an important cardiovascular autonomic component and contribute to orthostatic tolerance. Because space-induced vestibular dysfunction may contribute to the orthostatic intolerance experienced by astronauts returning to Earth, understanding vestibular/autonomic interactions can help develop more effective countermeasures to alleviate space motion sickness and post-flight orthostatic intolerance.

We have solved technical problems related to blood pressure and respiratory monitoring during OVAR. Specifically, the OVAR chair has been fitted with the portapres device (a new small device for beat to beat blood pressure measurements) and the slip rings modified to transmit its signals. Software has been modified to accept signals from both the ISCAN device and the portapres. Pilot trials of rotation during tilt have been successfully carried out, confirming our ability to carry out these studies.

Understanding vestibular autonomic interactions should result in the development of more effective therapies for motion sickness and orthostatic intolerance, two common and very disabling problems.

Assessment of the Effects of Chronic Microgravity on Ventricular Mass by Three-Dimensional Echocardiography

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No Co-Is Assigned to this Task

Funding:

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Post-Doctoral Associates: 0

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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Our objectives are: 1) to assess the effects of chronic microgravity on cardiac mass (myocardial volume) by three-dimensional echocardiography, and 2) to provide to NASA a space-capable three-dimensional ultrasound imaging system designed for accurate, quantitative measurements of organ volume and surface area for use by ourselves and other investigators.

To study the mechanism of cardiovascular adaptation of ventricular mass to chronic microgravity it is essential to have a means to accurately measure change of ventricular mass in individuals. This capacity has not previously been available. Three-dimensional echocardiography has been developed and proven to be methodologically superior to two-dimensional echocardiography for measurement of left ventricular volume, mass, surface area, and ejection fraction. Recent work indicates that it is able to accurately measure mass change in individuals, whereas previous methods have been validated only for populations. Previous work suggests that cardiac mass may decrease in microgravity. It may contribute to post-flight orthostatic intolerance and decreased exercise capacity. The time course and degree of decrease of cardiac mass are unknown due to lack of adequate, accurate available data for long-duration space flights. Adequate interventions and countermeasures cannot be evaluated until these data are available. Inflight assessment of cardiac mass will permit evaluation and alteration of countermeasures during the course of long-duration space flights.

We hypothesize that: 1) left ventricular adaptive changes to chronic microgravity results in 1) decreases of myocardial mass proportional to decreases in circulating blood volume, and 2) decreases attributable to decreased cardiac work, and these changes are reversible over extended periods of time on return to Earth gravity. Three-dimensional echocardiography will be used to obtain pre-flight, in-flight and post-flight data sets for reconstruction of the ventricle and computation of left ventricular volume, mass, and function. Paired T-test and repeated measured analysis of variance will be used to determine if significant change of these parameters has occurred. It is anticipated that the data will show, upon entry into microgravity, an initial increase in myocardial volume, then a rapid adaptation, normalization, and then a gradual decrease of myocardial volume to a new lower level of homeostasis with no further significant change in ventricular volume, mass, or function on long-duration space flight. After return to Earth gravity, we expect the data to show that there is a decrease in chamber and myocardial volume, then rapid adaptation and normalization of ventricular volume and a slow return

of mass to pre-flight values. Confirmation of our hypothesis will assure that astronauts will not suffer any permanent adverse effect on left ventricular function or mass on long duration space flight.

To facilitate development of a 3D ultrasound scanner for use in the International Space Station, a comparative study of spatial locators is being undertaken. Two established types of locators, acoustic and electro-magnetic, are being evaluated by determination of their accuracy locating the position and orientation of images when implemented into a three-dimensional echocardiographic system. Images of special three-dimensional calibration phantoms have been obtained and the volumes of the phantoms are being computed and compared to physically determined values. These systems will then be compared in the presence of a metallic, conducting environment to determine the boundary conditions defining interference with accurate measurement of volume, as might be experienced in the ISS. These comparisons are currently underway and will be reported subsequent to completion and analysis.

The long term benefits of this work will be to provide a better quantitative ultrasound imaging system for the International Space Station. Use of this instrument in the space station will provide a better quantitative understanding of the effect of weightlessness on many biological systems, but especially on the effects of microgravity on atrophy of the left ventricle.

Adaptation in Artificial Gravity Environments

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Funding:

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Task Description:

The objective of the proposed research is to provide a technical base for evaluating the feasibility of a rotating "artificial gravity" environment for long-duration space missions. We have previously demonstrated that Coriolis forces generated during rotation at 10 rpm disrupt head and arm movements but adaptation is possible. Here we will study 1) the rotation rates up to which adaptation is possible, 2) whether measurements of disruptions caused by rotation and subsequent adaptation in 1-G underestimate or overestimate the effects to be expected during rotation in environments with a background force level less than 1-G, and 3) how the magnitude and orientation of the background force affect retention and transfer of adaptation to rotation. Our studies will: 1) result in recommendations regarding design criteria for artificial gravity environments; 2) provide sound scientific reasons for establishing confidence limits on the recommendation; 3) provide a basis for designing preadaptation procedures to alleviate expected problems in a rotating space vehicle; and 4) enhance basic understanding of spatial orientation on Earth.

Our goal is to understand how Coriolis forces that are generated by body movements in a rotating environment disrupt movement coordination and how to alleviate or prevent these disruptions. We have found that: 1) Coriolis forces disrupt the paths and endpoints of goal directed movements; 2) subjects allowed repeated movements rapidly adapt, even in the absence of visual feedback about their reaching accuracy; 3) partial intermanual transfer of adaptation to Coriolis forces occurs; endpoint adaptation, but not path adaptation, transfers; and 4) subjects allowed visual feedback about reaching accuracy show the same initial magnitudes of path and endpoint deviations but adapt more rapidly than subjects denied visual feedback.

Work completed in FY97 shows that: 1) significant deviations of reaching movements occur with speeds of the rotating room as low as 5 rpm; 2) the size of perturbations of reaching movements increases with room rotation speeds from 2 to 15 rpm but plateaus between 15 and 20 rpm; 3) complete adaptation at 20 rpm is possible within 20 movements; 4) subjects exposed to Coriolis forces in the 0g phase of parabolic flight show less deviation of the trajectory curvature of reaching movements owing to a complete lack of inflection back toward the target after deviation from the intended path in the initial part of a reach; 5) stationary subjects exposed to virtual self-rotation make reaching errors that indicate they are expecting Coriolis forces which are actually absent; and 6) leg and head movements are also deviated by Coriolis forces and both show rapid adaptation.

Our current work on adaptive changes in head movement control points to neck proprioceptive as well as vestibular signals being a key factor in the disorientation and motion sickness elicited by head movements

during passive body rotation. We had earlier shown that simply altering the effective inertial mass of the head makes voluntary head movements provocative. These findings have significance for understanding the etiology of space motion sickness and motion sickness on Earth. They also have direct significance for understanding why cybersickness occurs in virtual environments. Almost all situations in which motion sickness occurs involve alterations in the normal patterning of eye and head movement control in relation to proprioceptive and vestibular feedback.

FY97 Publications, Presentations, and Other Accomplishments:

Bortolami, S., DiZio, P., and Lackner, J.R. "Homeomorphic eye motor control model for three dimensional saccade dynamics." Soc. Neurosci. Abstr., 23, 1560 (1997).

Cohn, J., DiZio, P., and Lackner, J.R. "Reaching errors depend on speed of illusory self-rotation: Automatic, graded pre-planning of compensations for expected Coriolis force perturbations." Soc. Neurosci. Abstr., 23, 2089 (1997).

Cohn, J.V., DiZio, P., and Lackner, J.R. "Reaching movements during illusory self-rotation show compensation for expected Coriolis forces." Soc. Neurosci. Abstr., 169, 19 (1996).

DiZio, P. and Lackner, J.R. "Circumventing side effects of immersive virtual environments" in "Advances in Human Factors/Ergonomics." Edited by: Smith, M.J., Salvendy, G., and Koubek, R.J. Elsevier, Amsterdam, Vol. 21 Design of Computing Systems, pp 893-897 (1997).

DiZio, P. and Lackner, J.R. "Deviations and rapid adaptation of leg movements perturbed by Coriolis forces in a rotating room." Soc. Neurosci. Abstr., 23, 1562 (1997).

DiZio, P. and Lackner, J.R. "Reaching trajectory and endpoint errors induced by Coriolis force perturbations in labyrinthine-defective subjects." Soc. Neurosci. Abstr., 169, 18 (1996).

Easton, D.E., Greene, A.J., DiZio, P., Lackner, J.R. "Auditory cues for orientation and postural control in sighted and congenitally blind people." Exp. Brain Res. (In Press).

Jeka, J.J., Easton, R.D., Bentzen, B.L., and Lackner, J.R. "Haptic cues for orientation and postural control in sighted and blind individuals." Perception & Psychophysics, 58(3), 409-423 (1996).

Jeka, J.J., Oie, K.S., Henson, E.M., Dijkstra, T.M.H., Schoner, G., and Lackner, J.R. "Somatosensory coupling to postural sway velocity." Soc. Neurosci. Abstr., 670, 11 (1996).

Lackner, J.R. "Motion sickness" in "Encyclopedia of Neuroscience." Edited by: Adelman, G. and Smith, B. Amsterdam: Elsevier Science, 2nd edition, CD-ROM version (1997).

Lackner, J.R. and DiZio, P. "Adaptation in a rotating artificial gravity environment." Brain Res. (In Press).

Lackner, J.R. and DiZio, P. "Combined influences of gravito-inertial force level and visual field pitch on visually perceived eye level." J. Vestib. Res., 7(2), 1-12 (1997).

Lackner, J.R. and DiZio, P. "Motor function in microgravity: Movement in weightlessness." Curr. Op. Neurobiol., 6, 744-750 (1996).

Lackner, J.R. and DiZio, P. "Sensory motor coordination in an artificial gravity environment." ASGSB Bull. (In Press).

Lackner, J.R. and DiZio, P. "Spatial orientation as a component of presence: Insights gained from non-terrestrial environments." Presence (In Press).

Lackner, J.R. and DiZio, P. "The role of reafference in recalibration of limb movement control and locomotion." *J. Vestib. Res.*, 7(5), 381-391 (1997).

Lackner, J.R., DiZio, P., Jeka, J.J., and Rabin E. "Fingertip contact suppresses the destabilizing effects of leg muscle vibration." *Soc. Neurosci. Abstr.*, 641, 8 (1996).

Leslie, K.R., Stickgold, R., DiZio, P., Lackner, J.R., and Hobson, J.A. "The effect of optokinetic stimulation on daytime sleepiness." *Archives Italiennes de Biologie*, 135, 1-10 (1997).

Money, K., Lackner, J.R., and Cheung, R. "The autonomic nervous system and motion sickness" in "Vestibular Autonomic Regulation." Edited by: Yates, B.J. and Miller, A.D. CRC Press (1996).

Rabin, E., DiZio, P., Bortolami, S., and Lackner, J.R. "Haptic stabilization of posture: Influence of fingertip contact location in relation to axis of instability." *Soc. Neurosci. Abstr.*, 23, 1561 (1997).

Motor Adaptation to Coriolis and Contact Forces

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Funding:

UPN/Project Identification: 199-16-17-05

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 8

Post-Doctoral Associates: 3

FY 1997 Funding: \$200,257

Task Description:

A rotating space vehicle could be used to generate "artificial gravity" during long-duration missions, but this would have side effects. Movements made during body rotation would generate transient Coriolis forces that act perpendicular to both the rotation axis and the movement direction. We have found that such Coriolis forces initially deviate the trajectories and endpoints of reaching movements but adaptation occurs to restore accuracy if exposure continues. The patterns of movement deviation generated by Coriolis forces differ from what has been observed when movements are perturbed by external, local contact forces of comparable timing and magnitude. This implies that cutaneous contact cues are critical in the control and monitoring of movement endpoint, trajectory, and adaptation. Our new goal is to investigate the conjoint influence on reaching and adaptation of cutaneous sensory signals, proprioceptors, and efferent commands. We will measure the effect on reaching movements of exposure to contact force perturbations, non-contact Coriolis forces and combinations of the two. Acquisition, retention, and transfer of adaptation will also be studied. The results will allow us to refine our model of the adaptation, planning, and execution of reaching movements. This will provide a basis for anticipating and solving potential performance problems in a rotating artificial gravity environment.

Our previous work showed that: 1) Coriolis forces generated by voluntary reaching movements in a rotating artificial gravity environment deviate the endpoints of those movements and make formerly straight paths curved; 2) adaptation is possible within 20 reaching movements during rotation at 10 rpm; 3) endpoint and path adaptation are complete and rapid if contact cues are permitted between fingertip and target surface but only curvature adapts fully if such contact is denied; 4) individuals lacking somatosensation due to neuropathy of large myelinated sensory fibers show larger deviations of reaching due to Coriolis forces, they also show some straightening of their movement trajectories with practice; 5) congenitally blind subjects adapt their movement trajectories and endpoints to Coriolis force perturbations as rapidly and fully as normal subjects tested blindfolded; and 6) labyrinthine defective subjects show diminished ability, relative to normal subjects, to adapt to Coriolis force perturbations of their reaching movements.

Our new experiments are being performed with a low inertia torque motor powered 3-dimensional manipulandum to investigate how contact force perturbations affect reaching movements. The manipulandum is programmed to apply to the hand a pattern of force that is identical to the Coriolis force on the hand in the rotating room. Reaching movements made under these conditions are deviated relative to reaches made with the unpowered manipulandum attached to the hand (or held in the hand). The endpoints and trajectory curvature errors are

significant when the power is first turned on, but subjects adapt with additional chances to reach. The adaptation is slower and less complete than adaptation to the non-contacting Coriolis forces generated in the rotating room. Mirror-image aftereffects of endpoint and trajectory are present after adaptation is achieved and the manipulandum is turned off, if it stays attached to the arm. If the manipulandum is removed and the arm is completely free after adaptation then no aftereffects are present and the movements are accurate. These findings demonstrate that equilibrium point theories of movement control are incorrect and that the presence or absence of contact forces is a critical factor determining the form and rate of adaptation to perturbations of movement.

Our work on the role of somatosensation and proprioception in adaptive motor control has led to a technique for enhancing postural control. Contact of the index finger with a stable surface at force levels far too low to provide any mechanical stabilization greatly stabilizes the body by providing cutaneous and proprioceptive cues about body sway. By minimizing changes in these signals, individuals stabilize their bodies. We have found that labyrinthine defective subjects who cannot stand for more than a few seconds without support can perform nearly as well as normal subjects when allowed fingertip contact. Analyses show that the subjects without functioning labyrinths are as stable with light touch in darkness as normal individuals in the dark without fingertip contact cues, indicating that sensory cues from the finger are superior to labyrinthine signals for minimizing sway. These studies provide new avenues for developing rehabilitation and training programs for individuals with loss of labyrinthine function and other types of balance disorders.

FY97 Publications, Presentations, and Other Accomplishments:

Bortolami, S., DiZio, P., and Lackner, J.R. "Homeomorphic eye motor control model for three dimensional saccade dynamics." *Soc. Neurosci. Abstr.*, 23, 1560 (1997).

Cohn, J., DiZio, P., and Lackner, J.R. "Reaching errors depend on speed of illusory self-rotation: Automatic, graded pre-planning of compensations for expected Coriolis force perturbations." *Soc. Neurosci. Abstr.*, 23, 2089 (1997).

Cohn, J.V., DiZio, P., and Lackner, J.R. "Reaching movements during illusory self-rotation show compensation for expected Coriolis forces." *Soc. Neurosci. Abstr.*, 169, 19 (1996).

DiZio, P. and Lackner, J.R. "Circumventing side effects of immersive virtual environments" in "Advances in Human Factors/Ergonomics." Edited by: Smith, M.J., Salvendy, G., and Koubek, R.J. Elsevier, Amsterdam, Vol. 21 Design of Computing Systems, pp 893-897 (1997).

DiZio, P. and Lackner, J.R. "Deviations and rapid adaptation of leg movements perturbed by Coriolis forces in a rotating room." *Soc. Neurosci. Abstr.*, 23, 1562 (1997).

DiZio, P. and Lackner, J.R. "Reaching trajectory and endpoint errors induced by Coriolis force perturbations in labyrinthine-defective subjects." *Soc. Neurosci. Abstr.*, 169, 18 (1996).

Easton, D.E., Greene, A.J., DiZio, P., and Lackner, J.R. "Auditory cues for orientation and postural control in sighted and congenitally blind people." *Exp. Brain Res.* (In Press).

Jeka, J.J., Easton, R.D., Bentzen, B.L., and Lackner, J.R. "Haptic cues for orientation and postural control in sighted and blind individuals." *Perception & Psychophysics*, 58(3), 409-423 (1996).

Jeka, J.J., Oie, K.S., Henson, E.M., Dijkstra, T.M.H., Schoner, G., and Lackner, J.R. "Somatosensory coupling to postural sway velocity." *Soc. Neurosci. Abstr.*, 670, 11 (1996).

Lackner, J.R. "Motion sickness" in "Encyclopedia of Neuroscience." Edited by: Adelman, G. and Smith, B. Amsterdam: Elsevier Science, 2nd edition, CD-ROM version (1997).

Lackner, J.R. and DiZio, P. "Adaptation in a rotating artificial gravity environment." *Brain Res.* (In Press).

Lackner, J.R. and DiZio, P. "Combined influences of gravito-inertial force level and visual field pitch on visually perceived eye level." *J. Vestib. Res.*, 7(2), 1-12 (1997).

Lackner, J.R. and DiZio, P. "Motor function in microgravity: movement in weightlessness." *Curr. Op. Neurobiol.*, 6, 744-750 (1996).

Lackner, J.R. and DiZio, P. "Sensory motor coordination in an artificial gravity environment." *ASGSB Bull.* (In Press).

Lackner, J.R., and DiZio, P. "Spatial orientation as a component of presence: Insights gained from non-terrestrial environments." *Presence* (In Press).

Lackner, J.R., and DiZio, P. "The role of reafference in recalibration of limb movement control and locomotion." *J. Vestib. Res.*, 7(5), 381-391 (1997).

Lackner, J.R., DiZio, P., Jeka, J.J., and Rabin, E. "Fingertip contact suppresses the destabilizing effects of leg muscle vibration." *Soc. Neurosci. Abstr.*, 641, 8 (1996).

Leslie, K.R., Stickgold, R., DiZio, P., Lackner, J.R., and Hobson, J.A. "The effect of optokinetic stimulation on daytime sleepiness." *Archives Italiennes de Biologie*, 135, 1-10 (1997).

Money, K., Lackner, J.R., and Cheung, R. "The autonomic nervous system and motion sickness" in "Vestibular Autonomic Regulation." Edited by: Yates, B.J. and Miller, A.D. CRC Press (1996).

Rabin, E., DiZio, P., Bortolami, S., and Lackner, J.R. "Haptic stabilization of posture: Influence of fingertip contact location in relation to axis of instability." *Soc. Neurosci. Abstr.*, 23, 1561 (1997).

Slow Rotating Room

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-97-17-05

Solicitation: not available

Initial Funding Date: 1995

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$65,000

Joint Agency Participation: NIH

Task Description:

We make access to our slow rotation room facility open to members of the scientific community with joint support from NASA and NIH. All on-board experimentation has to be conducted by principal investigators or senior scientists of the visiting investigation team; no unsupervised students are permitted to run experiments. We provide space and power, and the visiting team provides apparatus, supplies, and personnel. In addition to passing NASA/NIH peer review, any proposal for experimental work in the slow rotation room has to meet feasibility requirements in terms of requested force profiles and safety requirements to ensure that subject safety is preserved. All personnel and equipment involved in the slow rotation room experiments have to meet the same safety requirements as for participation in parabolic flights on NASA's KC-135 aircraft because the potential stresses can be comparable. A written statement describing the proposed protocol, resource requirements, and safety matters must meet the approval of the Director and Associate Director of the Graybiel Laboratory. The Brandeis Committee for the Protection of Human Subjects must approve all procedures involving human subjects and experimenters.

Eight outside investigator projects were conducted in the rotating room during FY97. Two additional outside investigator proposals have been prepared with our cooperation and been submitted to funding agencies for support to use the rotating room.

The rotating room is a powerful tool for investigating the physiology of adaptation to Earth's gravity as well as for predicting and solving problems related to space travel. It provides a ground-based unusual force environment in which to observe plants and animals. Abnormalities observed in this environment point to the need for theories about processes and phenomena that are hidden in normal environments.

Spatially Oriented Database for Digital Brain Images [Human Brain Project]

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Ed Herskovitz; Johns Hopkins Medical Institutions
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Funding:

UPN/Project Identification: not applicable

Solicitation: not available

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: not available

Joint Agency Participation: NIH and Human Brain Project

Task Description:

The overall goal of this project is the conceptual development and prototype implementation of a database methodology that supports the archiving and statistical investigation of large numbers and types of brain images. The specific aims of the study are: 1) to develop a morphologically factored image representation (MFIR) system that allows improved comparison of brain images, 2) to develop a Brain Image Database (BRAID) that supports novel statistical analyses of image data sets, and 3) to evaluate the database by applying it to both simulated data and to real data from 3 current brain imaging studies.

The MFIR is based on a nonlinear registration of an image to a standard atlas to create a morphologically normalized signal component and a morphological variation component, represented as a displacement vector field in atlas coordinates. The BRAID will implement storage, query and statistical operations on the MFIR components. The BRAID will be validated by testing its ability to recover known correlations from simulated data, and applied to the analysis of data from several collaborating epidemiological studies. The applications will test the system's ability to identify brain structure/function correlations from lesion/deficit data derived from stroke and injury, and its ability to identify patterns of morphological change in brain anatomy with age, and correlate these with functional data. Stroke data will be provided by the Cardiovascular Health Study, a National Heart, Lung, and Blood Institute sponsored project that is collecting extensive prospective demographic, functional, and brain Magnetic Resonance Imaging data on over 3,600 participants. Injury data will be collected by the Psychopathology of Frontal Lobe Injury in Childhood study, which is collecting brain MRI and extensive psychiatric/functional data on 100 children with traumatic brain injuries. Aging-related morphological and functional change data will be supplied by the Baltimore Longitudinal Study on Aging, which follows 180 patients over a nine year period and performs MRI and Positron Emission Tomography scans along with neurofunctional evaluations, on an annual basis. The newly developed database is intended to be flexible in terms of acceptable data types, robust in its querying mechanisms, and extendable to other laboratories, thus providing the basis of a future broad-based, multi-institutional brain informatics network.

This research will provide insights into the localization of brain functions and the effects of stroke on brain function, and on the changes that occur in the structure and function of the brain with age. It will also evaluate a novel technology, extensible object/relational DBMSs, in an application which bears some relationship to geographic information systems and remote sensing databases. The object/relational technology enables an integration of data type-specific operations with general purpose relational ones. When the datatypes are spatially oriented, the result will hopefully be more powerful spatial database technologies.

The Role of Cardiac Mechanics in Blood Pressure Regulation During Orthostatic Stress: The Effect of Duration of Exposure to Simulated Microgravity

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Funding:

UPN/Project Identification: 199-14-17-20
Initial Funding Date: 1997
Students Funded Under Research: 0
FY 1997 Funding: \$139,000

Solicitation: 96-OLMSA-01
Expiration: 2000
Post-Doctoral Associates: 1

Task Description:

Changes in cardiac mechanics play an essential role in determining the adaptive response of the cardiovascular system to microgravity, and contribute to orthostatic intolerance upon return to a 1-G environment. The objective of this proposal is to investigate the mechanisms by which changes in cardiac structure (mass) and function (pressure-volume relationships) during acute (hours), short-term (weeks), and long-term (months) exposure to simulated microgravity affect the regulation of blood pressure during orthostatic stress. Acute studies: We hypothesize that acutely, the principal adaptation that leads to the clinical problem of orthostatic intolerance is a reduction in plasma volume that impairs cardiac filling. We propose to counteract this problem by optimum volume loading with intravenous dextran, thereby restoring orthostatic tolerance. Short term studies: In contrast to acute microgravity, recent studies in the PI's lab suggest that 2 weeks of head-down bedrest leads to both a reduction in plasma volume and a decrease in left ventricular (LV) distensibility, possibly due to a reduction in LV mass as measured by echocardiography. We propose to: a) identify the mechanism for this adaptation by using magnetic resonance imaging to confirm a reduction in LV mass, and b) determine the relative importance of reduced cardiac distensibility versus hypovolemia by completing 2 weeks of head-down bedrest in three additional groups of subjects: 1) exercise training alone to normalize cardiac distensibility; 2) volume infusion alone to normalize cardiac filling pressure; and 3) exercise training plus volume infusion to normalize both. We hypothesize that only the latter will completely normalize orthostatic tolerance after short term head-down bed rest. Summary: By combining direct measures of cardiac mass and pressure-volume characteristics with a novel approach to analyzing the integrated circulatory response to orthostatic stress using transfer function analysis, we expect to: 1) define the relative importance of changes in plasma volume, cardiac mass and compliance in the adaptation to microgravity; 2) identify specific countermeasure strategies based on well-defined physiology; and 3) gain insight into the mechanism for the acute hemodynamic response to microgravity.

Funding for this project was received in August 1997, so there has been little time for substantive progress to date. We have focused on obtaining approval from the General Clinical Research Center at UT Southwestern for support of the bed rest studies, and this approval has been granted. We have been working on detailed protocol

development, and piloting of the specific protocol to be used in the acute bed rest/volume infusion studies, and these are underway. We plan to complete specific aim #1 by the end of July, 1998.

This study will have both scientific and operational ramifications. From a scientific standpoint, the mechanism of an important clinical problem - that of bed rest "deconditioning" will be elucidated and attributable to cardiac mechanical factors. This data may be relevant for patients after acute myocardial infarction or with congestive heart failure, in whom cardiac stiffness is radically altered by disease processes. From an operational perspective, if orthostatic intolerance after acute head-down tilt bed rest can be completely reversed with optimum volume loading, then this result would focus future studies specifically on strategies to restore plasma and cardiac volume to normal after space flight. For example, other strategies for oral rehydration including glycerol ingestion or adjunctive pharmacologic agents such as DDAVP, fludrocortisone, or venodilators should then be aggressively pursued. However if even optimum volume expansion does not restore orthostatic tolerance, then other countermeasures focusing on facilitating an increase in vascular resistance might be more productive.

In addition, this study will be the first to demonstrate conclusively the presence of cardiac atrophy under physiological conditions in humans, and therefore will provide new basic information regarding the plasticity of cardiac adaptation. From an operational perspective, if atrophy is demonstrated and confirmed to be an important contributor to orthostatic intolerance, then specific countermeasures can be designed to minimize this response, both in terms of space flight and deconditioning secondary to chronic disease.

Altered Brain Vasoregulation in Orthostatic Intolerance

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J.D. Schmelzer, B.S.
Ben McPhee, B.S.

Funding:

UPN/Project Identification: 199-14-17-11
Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$247,238

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 4

Task Description:

The overall objective of this research is to gain insights into microgravity-associated orthostatic intolerance (MOI) by studying the alterations in cerebral vasoregulation and the effects on brain oxygenation during tilt-up in patients with orthostatic intolerance, manifest as orthostatic tachycardia and lightheadedness. The justification for the study resides in 1) the close similarity in symptoms and possible mechanisms in patients with orthostatic intolerance and MOI; 2) the early dynamic alterations in cerebral vasoregulation, perhaps preceding changes in BP and heart rate; 3) the paradoxical cerebrovascular responses to tilt-up, and isoproterenol infusion, reacting with vasoconstriction rather than vasodilatation; 4) the need for evaluating the effect of vasoconstriction on the brain using the EEG; and 5) the preliminary results suggest that it might be possible to evaluate brain stem autonomic rhythms using the novel approach of time-frequency spectral analysis of amplitude modulation of the EEG.

We have made excellent progress in FY97 in all specific aims of the current project. On studies on brain vasoregulation, we defined the different patterns of vasoregulation in patients with autonomic failure (normal; expanded; autoregulatory failure with flat flow:pressure slopes; autoregulatory failure with steep flow:pressure slopes). Patients with orthostatic intolerance develop symptoms because of a reduction in cerebral perfusion due to hypocapnia. We have completed approximately 70% of EEG recordings and are currently undertaking the major task of data analysis (time-frequency analysis; amplitude modulation of EEG). Studies on the efficacy of resistance training are approximately 70% completed.

The focus of our research is uniquely situated in that we are evaluating an illness that afflicts humans on Earth, but by mechanisms that are likely to be identical to those that cause orthostatic intolerance with extended periods in space. The project is specifically focused on alleviating the problem of orthostatic intolerance that develops with microgravity, deconditioning, and prolonged bedrest. It evaluates the mechanisms, including brain mechanisms, and couples that with an evaluation of methods of treating the problem. We approach treatment

with evaluating resistance training coupled with physical countermeasures. The studies of the brain, and in particular the brain stem ultra-slow rhythms, detectable on amplitude modulation of the EEG, may provide important understanding of brain-stem mechanisms in regulating BP. The studies, by attempting to unify mechanisms of orthostatic intolerance on Earth and in space, provide a self-reinforcing approach to link space and Earth. The clinical applications of the research are potentially highly significant. It may result in a new way to treat orthostatic intolerance, as well as new methods to recognize it. The approach that we have adopted is unique in several respects. We have developed new algorithms, hitherto unavailable, to evaluate signals (time-frequency analysis, amplitude modulation of the EEG), and a combined approach in treatment of using physical countermeasures and resistance training.

FY97 Publications, Presentations, and Other Accomplishments:

Bharucha, A.E., Camilleri, M., Novak, V., Zinsmeister, A.R., and Low, P.A. "Alpha2 adrenergic modulation of colonic tone during hyperventilation." (Abstract) *Gastroenterol.*, 112 (4 Suppl), A699 (April, 1997).

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Low, P.A. "Laboratory evaluation of autonomic function" in "Clinical Autonomic Disorders: Evaluation and Management." Edited by: Low, P.A. New York: Lippincott-Raven, pp 179-208 (1997).

Low, P.A. "The effect of aging on the autonomic nervous system" in "Clinical Autonomic Disorders: Evaluation and Management." Edited by: Low, P.A. New York: Lippincott-Raven, pp 161-175 (1997).

Low, P.A., Ahlskog, J.E., Petersen, R.C., Waring, S.C., Esteban-Santillan, C., and Kurland, L.T. "Autonomic failure in Guamanian neurodegenerative disease." *Neurology* (In Press).

Low, P.A. and McLeod, J.G. "Autonomic neuropathies" in "Clinical Autonomic Disorders: Evaluation and Management." Edited by: Low, P.A. New York: Lippincott-Raven, pp 463-486 (1997).

Low, P.A. and Schondorf, R. "Postural tachycardia syndrome" in "Primer on the Autonomic Nervous System." Edited by: Robertson, D., Low, P.A., and Polinsky, R.J. San Diego: Academic Press, pp 279-283 (1996).

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Physiological Transport Responses to High Intensity Exercise and Hydrostatic Pressure Gradients in Humans

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Funding:

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Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The circulatory adjustments to orthostatic stress and exercise training include increased transfer of fluid between the extravascular and vascular compartments. Following intense exercise, plasma volume is returned to its control level within 2 hours, despite a significant (>800 g) deficit in total body water because of a translocation of proteins into the vascular compartment. Preliminary observations in our laboratory demonstrate a smaller translocation of protein and fluid into the vascular compartment during recovery from exercise in the supine compared to the sitting position. Thus, hydrostatic pressure gradients appear to alter the exercise-stimulated protein and fluid transport. The mechanism by which hydrostatic pressure gradients influence the movement of fluid and protein between extra- and intravascular compartments is unclear. The purpose of this proposal is to examine the mechanism by which high-intensity exercise induces a net transfer of fluid and protein into the vascular space and to determine how these processes are influenced by changes in hydrostatic pressure gradients.

The specific aims of this project are to: 1) Characterize the movement of albumin and fluid that contributes to a selective expansion of plasma volume following intense exercise. We will quantify the Starling factors which contribute to the movement of fluid into the vascular compartment, examine changes in plasma and interstitial fluid (ISF) colloid osmotic pressures in skin and muscle which provide the driving forces for fluid movement and lymphatic transport of protein into the vascular space; 2) Examine the influence of hydrostatic pressure gradients on the movement of albumin and fluid following intense exercise. We anticipate that changes in hydrostatic pressure gradients associated with movement from the upright to the supine posture will attenuate albumin and fluid transport; and 3) Examine movement of fluid and albumin between extravascular and intravascular compartments during saline loading following high intensity exercise. We will measure fluid retention following intense exercise while loading the vascular compartment with a bolus saline infusion. In addition, we will examine renal function and endocrine responses to the volume load, allowing us to identify the renal contribution to this response.

Over the past year, we have prepared several manuscripts for publication based upon the data collected from the previous two years of work on our NASA grant. These manuscripts are specifically related to mechanisms of

plasma volume expansion following intense exercise and factors which modulate this response. These manuscripts are listed in the bibliography.

In the laboratory, we have focused our research efforts on two specific issues. We continue to evaluate the impact of posture on the translocation of albumin from extravascular to intravascular stores. A bolus saline infusion was used to increase capillary filtration and increase lymphatic flow. The bolus saline infusion increased plasma volume by 3.54 ± 0.59 and 4.84 ± 0.54 ml/kg body wt. in the upright and supine posture, respectively. The transcapillary escape rate of albumin, measured by the disappearance rate of albumin-bound Evan's blue dye, was similar in both postures ($\approx 7.6\%/h$). Following the bolus saline infusion, plasma protein content and albumin content increased (0.11 ± 0.03 and 0.06 ± 0.02 g/body wt., respectively) in the upright but not the supine posture. The translocation of protein into the vascular space following bolus saline infusion reflects increased lymph return of protein presumably due to decreased central venous pressure and a reduction in the afterload for lymph return in the upright posture.

The second area of interest this year has been the contribution of renal adjustments to the process of exercise-induced hypervolemia. In this series of experiments, we also used a bolus saline infusion; however, in this case we were interested in challenging the kidney with a salt and water load. We examined the renal sodium and water handling before and 24 hours following exercise-induced hypervolemia in order to identify the specific renal mechanism(s) involved in plasma volume expansion. In control conditions, a bolus saline load (15 ml/kg) increased glomerular filtration rate, filtered sodium load, lithium clearance, and proximal tubular sodium output. Exercise induced a 6.1% increase in plasma volume by twenty-four hours and in response to saline loading, we saw no significant change in glomerular filtration rate, filtered sodium load, lithium clearance, or proximal tubular sodium output. Plasma levels of aldosterone and distal tubular sodium reabsorption was similar before and after exercise. These data indicate that the sodium sparing occurs during exercise-induced hypervolemia and is mediated primarily by a decrease in glomerular filtration rate.

The forces responsible for the distribution of fluid between the vascular and interstitial fluid compartments are well defined (at 1-G), yet the mechanism by which these forces interact or respond to a variety of disturbances that eventually induce changes in the distribution of fluid is not well understood. Our research focuses on the basic biological process of physiological transport of fluid and albumin and how this process is altered by such disturbances such as intense exercise and/or changes in body posture (hydrostatic pressure gradients within the vascular compartment). Results from our studies will directly provide insight into the mechanism of plasma volume expansion. This insight should provide a focus for researchers in a variety of fields as they attempt to understand fluid dynamics under both normal (pregnancy) and disease (sepsis, congestive heart failure) states on Earth. In addition, we will be able to define how these biophysical principles (Starling forces) interact under conditions of exercise and simulated microgravity (supine posture) and thus define the impact of an exercise countermeasure on plasma volume expansion in space.

FY97 Publications, Presentations, and Other Accomplishments:

Haskell, A., Gillen, C.M., Mack, G.W., and Nadel, E.R. "Albumin infusion in humans does not model exercise induced hypervolemia after twenty-four hours." *Acta Physiol. Scand.* (In Press).

Haskell, A., Nadel, E.R., Stachenfeld, N.S., Nagashima, K., and Mack, G.W. "Forces governing transcapillary protein and fluid flux in human muscle and skin twenty-four hour after intense exercise." *J. Appl. Physiol.*, 83(2), 407-413 (1997).

Haskell, A., Stachenfeld, N.S., Nagashima, K., and Mack, G.W. "Capillary pressure in the human forearm during exercise induced hypervolemia." *J. Appl. Physiol.* (In Press).

Mack, G.W., Yang, R., and Haskell, A. "Influence of hydrostatic pressure gradients on regulation of plasma volume following exercise." *J. Appl. Physiol.* (In Press).

Cardiac Valvuloseptal Morphogenesis

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 5 P01 HL52813-04 (NIH) Solicitation: not available
Initial Funding Date: 1994 Expiration: 1999
Students Funded Under Research: 0 Post-Doctoral Associates: 0
FY 1997 Funding: \$92,901
Joint Agency Participation: NIH/National Heart, Lung, and Blood Institute

Task Description:

One percent of newborn humans have some congenital heart defect, usually in the septation of the heart. The septation of the heart arises from a series of epithelial-mesenchymal transformations wherein epithelial cell adhesion and convert to migrating mesenchymal cells which finally differentiate into the septation of the heart. Despite this medical importance, little is known about the molecular bases for these processes. The long-term objective of these studies is to test the idea that molecules involved in cell adhesion regulate cell behavior via signal transduction cascades initiated by intermolecular interaction at the cell surface. Our current goal is to test the hypothesis that by altering the mechanical environmental in cells, a microgravity environment will affect cell adhesion and migration by altering the mechanochemical processes involved in signal transduction cascades and the formation and function of linkages between cell-surface proteins and the cytoskeleton. Indeed, in preliminary experiments microgravity caused mesenchymal cells to migrate in a totally novel pattern, in single file with close contact between neighboring cells rather than as individual cells in random orientation.

To test this hypothesis, the following specific aims will be performed: 1) Compare the distributions under unit gravity and microgravity of the cell surface and extracellular matrix proteins that mediate and regulate adhesion, F-actin, and proteins linking transmembrane adhesion molecules to F-actin. 2) Identify signal transduction mechanisms that are perturbed by microgravity leading to the altered behavior of mesenchymal cells. These experiments will use a variety of immunological, cell biological, and protein chemical methods. The results of these studies will help us better understand the biological consequences of prolonged space flight for astronauts and fetuses conceived in space. More important and relevant to the health of people on Earth, the observation that microgravity produces totally novel effects on mesenchymal cell migration makes microgravity a unique tool to probe the basic regulatory mechanisms that regulate the septation of the heart and go awry in congenial heart defects.

Molecular Mechanisms Regulating IGF-I Synthesis in Bone

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Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$139,557

Task Description:

Microgravity-induced osteopenia appears to be caused by uncoupled bone remodeling resulting from reduced mechanical stress. Currently, few details have emerged regarding signal transduction resulting from mechanical stress; however, prostaglandins of the E series (PGE) are believed to participate as local mediators of mechanical stress in bone. PGEs such as PGE₂ and PGE₁ elevate intracellular cAMP levels in many bone cell culture models, which serves to activate protein kinase A (PKA). *In vivo* parathyroid hormone (PTH) is the central calciotropic hormone in coupled bone remodeling. In osteoblasts, PTH stimulates cAMP and prostaglandin synthesis, and has a subsequent stimulatory effect on PKA activity. Both PTH and PGE₂ potently and rapidly elevate IGF-I synthesis by osteoblasts. This proposal seeks to determine the molecular mechanisms that regulate IGF-I expression in rat bone cells, by determining the regulatory sequences within IGF-I promoter 1 that influence basal and PGE₂ (cAMP) stimulated IGF-I expression; the influence of mechanical force (cyclic mechanical strain) on IGF-I promoter activity will be assessed and associated regulatory sequences determined.

This grant proposal has three specific aims. Specific Aim #1: Determine the DNA segments within promoter 1 of the rat IGF-I gene that confer sensitivity to stimulation by prostaglandin E₂ (PGE₂) in fetal rat osteoblasts (Ob); Specific Aim #2: Characterize nuclear protein factors from Ob cells that interact with basal and PGE₂ response elements within the IGF-I promoter; and Specific Aim #3: Determine if a cause and effect relationship exists between mechanical force, PGE₂ (and cAMP) induction and IGF-I promoter utilization.

As reported in FY96 Task Progress Report, Specific Aim #1 was accomplished, and the results were published in *Endocrinology* (McCarthy, T.L., M.J. Thomas, M. Centrella, and P. Rotwein, Regulation of insulin-like growth factor I transcription by cyclic 3,5 -monophosphate (cAMP) in fetal rat bone cells through an element within exon 1: Protein kinase A dependent control without consensus cAMP response elements. *Endocrinology* 136:3901-3908, 1995). Briefly, we localized a cis-acting promoter element(s) responsible for cAMP stimulated gene expression to the 5' untranslated region (UTR) of IGF-I exon 1, within a segment lacking a consensus cyclic AMP response element, and determined that PGE₂ influenced IGF-I promoter activation through protein kinase A (PKA) activation. DNase I footprinting of this 5' UTR of exon 1 demonstrated protected sequences at HS3A, HS3B, and HS3D, three of six DNA-protein binding sites previously characterized with rat liver nuclear

extracts. Of these three regions, only the HS3D binding site is located within the functionally identified PGE₂ responsive segment of IGF-I exon 1.

We next extended this observation, and identified the minimal sequence needed for inducible binding at the HS3D footprint region, as tested in the gel mobility shift assay using nuclear protein extracts prepared from control and PGE₂ treated osteoblast cultures, and transfection with mutant IGF-I promoter 1 constructs. This non-consensus cyclic response element (CRE) is 5'-CGCAATCG-3' and spans the +202 to +209 bp region of exon 1. Point and linker scanning mutations have been introduced into the HS3D footprint region, and both transient transfection and gel mobility shift analyses corroborate the importance of this sequence in PGE₂ stimulated IGF-I expression. These data were presented at the 10th International Congress of Endocrinology, and were published in *The Journal of Biological Chemistry* (1996) vol. 271, pp 21835-21841, in a manuscript entitled "Identification of the cAMP response element that controls transcriptional activation of the insulin-like growth factor-I gene by prostaglandin E₂ in osteoblasts".

We now report that we have successfully accomplished our goals set out in Specific Aim #2. Nuclear protein extracts prepared from control and PGE₂ treated primary rat osteoblast cultures were tested in gel mobility shift assays for binding to the novel CRE present in exon 1 at the HS3D footprint site. Initial characterization was accomplished by recovery of the PGE₂ induced gel shift bands, followed by UV cross-linking of ³²P-labeled oligonucleotide, to examine the relative molecular mass of the bound protein(s) on a denaturing SDS-PAGE. Results indicate a protein of <42 kDa binds to the HS3D site in nuclear extracts from PGE₂ treated cultures. Preliminary searching using the Wisconsin Sequence Analysis Package (GCG) sequence database indicated some homology with several viral promoter elements that have similarity with CAAT element binding protein (C/EBP) sites. Gel mobility supershift analysis was conducted using a cross-reactive antibody to C/EBP that recognizes multiple isoforms of this transcription factor. A supershift was observed, indicating binding of C/EBP to the HS3D site present in the synthetic ds oligonucleotide. Using a C/EBP δ specific antibody, we identified the reactive nuclear protein as the δ isoform of C/EBP. Additional studies employing rat C/EBP δ and C/EBP β expression vectors and co-transfection with IGF-I promoter constructs demonstrated augmentation of both basal and PGE₂ induced IGF-I promoter activity by C/EBP δ , and enhanced PGE₂ induced IGF-I promoter activation with C/EBP δ or C/EBP β co-transfection. These new data were presented as an oral presentation at the annual meeting of the Endocrine Society June, 1997 in Minneapolis, Minnesota. This observation was recently accepted for publication in *The Journal of Biological Chemistry* in a manuscript entitled "CCAAT/enhancer binding protein delta activates insulin-like growth factor-I gene transcription in osteoblasts: Identification of a novel cyclic AMP signaling pathway in bone."

Specific Aim #3 involves testing the effect of mechanical strain on the expression of IGF-I promoter activity. This line of research examines mechanical strain applied to our primary osteoblast cultures using an Flexercell[®] FX-3000. Cultures are plated on collagen coated flexible bottom cluster dishes, transfected with active IGF-I promoter construct (IGF1711b/Luc) when they achieve 60-75% confluence, grown to confluence, then tested for their response to low amplitude and low frequency mechanical strain of varied duration. Control cultures are plated on comparable plates, and experience no mechanical strain or are treated with an optimal dose (1 μ M) of PGE₂ to demonstrate a positive response to this IGF-I transcriptional activator (positive control). Preliminary data indicate a weak response to 0.1 Hz mechanical strain detectable within 6 hr of initiation. Further testing of varied amplitude, frequency, and duration are needed to optimize the response. Current efforts include an examination of transcript levels for IGF-I, C/EBP δ and C/EBP β , following mechanical strain protocols described above for promoter transfection studies. These studies are ongoing.

The high level of endogenous IGF-I synthesis by bone cells and its anabolic effects on bone indicate a major role for this factor in normal bone physiology. Locally produced IGFs are thought to participate in coupling bone formation to bone resorption. Therefore, it is important to understand the mechanisms bone cells utilize to regulate IGF-I activity. It is clear that IGF-I synthesis by osteoblasts is hormonally regulated. However, far less is presently known about the molecular mechanisms that regulate IGF-I expression. The loss of bone mass resulting in osteoporosis, seen in astronauts following exposure to microgravity and in older individuals, is thought to result from an imbalance between bone resorption and bone formation. In this vein, it is possible

that a decrease in IGF-I synthesis resulting from a decrease in mechanical stimuli (in microgravity, or extended bedrest due to illness), or changes in hormonal status (post-menopausal, in aging, or in microgravity) may occur and limit the amount of available biologically active IGF-I. Reduced IGF-I levels may in part be responsible for uncoupled bone remodeling.

The effects of microgravity may be influenced directly by locally produced agents (prostaglandins, growth factors), and long-term skeletal defects may result from the indirect effects of changes in hormonal status (and subsequent changes in local growth factor actions). These are contributing factors that may be common to various forms of osteoporosis and disuse osteopenia, and even the associated bone loss observed in cases of trauma and immobilization, such as in severely burned individuals. Therefore, a thorough understanding of the mechanisms that regulate IGF activity in skeletal tissue is crucial to develop a more complete picture of normal bone physiology and may provide the means to augment bone matrix synthesis and to minimize or reverse the bone loss that results from the debilitating effects of microgravity induced and other forms of osteoporosis.

FY97 Publications, Presentations, and Other Accomplishments:

McCarthy, T.L., Ji, C., Shu, H., Casinghino, S., Crothers, K., Rotwein, P., and Centrella, M. "17 β -estradiol potently suppresses cAMP induced insulin-like growth factor-I gene activation in primary rat osteoblast cultures." *J. Biol. Chem.*, 272, 18132-18139 (1997).

Umayahara, Y., Ji, C., Centrella, M., Rotwein, P., and McCarthy, T.L. "CCAAT/enhancer binding protein delta activates insulin-like growth factor-I gene transcription in osteoblasts: Identification of a novel cyclic AMP signaling pathway in bone." *J. Biol. Chem.* (In Press).

Environmental Constraints on Postural and Manual Control

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Funding:

UPN/Project Identification: 199-16-11-48

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: not available

Responsible NASA Center: Johnson Space Center

Task Description:

Extravehicular activity (EVA) is pivotal in supporting shuttle and space station operations, including maintenance, construction, and contingency tasks. A ground-based investigation has been conducted on the Precision Air-Bearing Floor (PABF) to provide information about mass-handling tasks that are characteristic of ongoing EVA (e.g., Hubble repair and retrofit missions) as well as planned EVA (e.g., space station assembly and maintenance). This investigation promotes a better understanding of the whole-body skill of extravehicular mass-handling and, thus, it can help crewmembers convey their knowledge and experience about EVA for the purposes of training and planning for future missions. The investigation also promotes a better understanding of simulator fidelity with respect to extravehicular mass-handling and, thus, it can lead to improvements or developments in simulators used to train and plan for future missions. Finally, the investigation has expedited the development of measurement techniques that can be used on-orbit for more rapid evaluation and more efficient debriefing of EVA.

Crewmembers have emphasized the importance of minimizing or otherwise controlling "cross coupling" forces at the ORU during EV mass-handling. They also have emphasized the importance of body stability. We believe that managing cross-coupling between orthogonal planes of postural motion is an important, albeit tacit, component of EVA skill. Furthermore, we believe such forces and motions are sufficiently subtle to be either damped out in ground-based simulators (e.g., WETF) that allow extravehicular mobility unit (EMU) motion in orthogonal body planes (e.g., sagittal and horizontal) or precluded entirely in other simulators (e.g., PABF). If we are correct, there would be little or no opportunity to learn this component of EVA skill on the ground. A "Yaw-Axis Cradle" (YAC) was developed for use with the EMU in the recumbent position on the PABF. The PABF-YAC allows us to investigate the skill of managing the postural mobility/stability tradeoff concurrently in orthogonal rotational axes (i.e., pitch and yaw).

We collected data in the PABF-YAC from EVA-experienced and EVA-inexperienced subjects in a simulation of an extravehicular battery-box replacement. Data were collected using force plates, accelerometers, and videographic methods. We developed unique analytical techniques to quantify the essential aspects of

mass-handling skill. An essential characteristic of such skill is the adaptive coupling within the ORU-crewmember-EMU-restraint complex and its control as a nested system. Another characteristic of mass-handling skill is the nesting of time scales for observation and control of both the ORU and the EMU. The spatial and temporal aspects of this nested control are unified in our anthropometrically valid methods for analyzing mass-handling performance and the associated whole-body coordination. We also have verified the operational validity of the experimental methods. Thus, recommendations about on-orbit applications will be appropriate.

We have determined that information about the coupling in the nested human-environment system is available in data collected with common instruments (e.g., video and force transducers). We have developed methods that reduce raw data from convenient instruments to task-relevant measures of postural-manual coupling. These measures are sufficiently robust to be applied in low-observability situations such as ORU-EMU coupling and, potentially, to be used on-orbit. The results have revealed that (a) the additional degree of freedom of mobility in the PABF-YAC has consequences for mass-handling; (b) postural motion in each degree of freedom influences the smoothness of ORU control; (c) the interaction between orthogonal planes of postural motion is such that it would be impossible, for example, to predict the effect of a pitch-axis perturbation on ORU motion without knowing the magnitude of concurrent yaw-axis motion; (d) postural configuration influences the postural stability; and (e) postural configuration influences the smoothness of ORU control.

These results are important because they reveal subtle patterns of body motion along with their task relevant consequences that cannot be learned in extant ground-based simulators. Based on our previous research on a variety of whole-body skills, and based on crewmember comments about this specific skill, we believe it is quite reasonable to assume that observation and control of these subtle patterns and their consequences is an essential and largely tacit component of EV mass-handling skill. It follows that improvements in simulator fidelity and in understanding the implications of crewmember comments can be greatly facilitated by the quantitative methods developed in this investigation or by conceptually similar analyses. In particular, it is important to develop EVA simulators that provide exposure to subtle patterns in the relationships among postural configuration, postural stability, and ORU control in order to support the acquisition of mass-handling skills that are used during EVA. Finally, these patterns can provide an appropriate behavioral basis for decisions about the placement of PFR or MFR during construction and maintenance of the International Space Station.

This research addresses the coordination of postural control and manual control. The skill of coordinating such nested body systems is relevant to most human-environment interactions. This skill is necessitated by upright posture and, arguably, it is the reason for upright posture. The adaptive-control-theoretic approach to coordination of nested systems in this research provides new insights into this basic human skill and into other basic biological processes that require detectability and stabilizability of nested biomechanical systems.

There are many constraints on human performance in EVA that are different in origin but similar in effect to constraints imposed on human performance on Earth. Such effects include: (a) reduced visibility due to inadequate illumination, contrast, and field of view; (b) reduced sense of orientation due to inadequate vestibular simulation; (c) reduced proprioceptive sensitivity due to inadequate stimulation of skin, joints and muscles; (d) reduced range of motion due to limitations on the joints; (e) inadequate strength relative to common task demands; (f) reduced support due to inadequate rigidity, extent, friction, or orientation of surfaces and restraints; and (g) inappropriate placement of objects to be seen and handled.

Constraints on interactions with the surroundings can be alleviated through the design of work environments that promote coordination between postural control and manual control or at least that allow postural adaptation to unusual conditions. This research seeks to understand this process of coordination along with the environmental and biological requirements for the associated skills. The results could have an impact to the extent that they lead to or suggest therapies, protocols, or assistive technologies that can alleviate problems imposed on the general skill of coordinating postural control and manual control.

Earth-based and non-NASA research on coordination of postural control and manual control can be leveraged in the investigation, and developing understanding, of human performance in EVA. Conversely, this NASA

research can inform non-NASA investigations about fundamental postural skills and constraints on their use and adaptability. Toward these ends, a complementary experiment has been conducted in collaboration with colleagues at the University of Massachusetts. These data were collected to evaluate the utility of exploring ORU "math properties" (i.e., handling qualities) in shirtsleeve conditions. This is important because it is an exercise that can be done quickly and informally by crewmembers on the PABF. This investigation also is important because it has resulted in a transfer of technology (or methods) from NASA to a research community in which they will be used to investigate motor disorders.

FY97 Publications, Presentations, and Other Accomplishments:

McDonald, P.V. "Mobility or stability? Understanding the effect of weightlessness on human performance." Colloquium at University of Minnesota, Minneapolis, MN (November, 1996).

McDonald, P.V., Riccio, G.E., Peters, B.T., Layne, C.S., and Bloomberg, J.J. "Understanding the skill of extravehicular mass handling." 12th Man in Space Symposium: The Future of Humans in Space, Washington, DC (June 8 - 13, 1997).

Peters, B.T., McDonald, P.V., Riccio, G.E., Layne, C.S., and Bloomberg, J.J. "Postural stability and mobility during extravehicular mass handling." 15th Annual Houston Conference on Biomedical Engineering Research, Houston, TX (February, 1997).

Riccio, G.E., McDonald, P.V., Peters, B.T., Layne, C.S., and Bloomberg, J.J. Understanding skill in EVA mass handling: Part I: Theoretical & operational foundations. NASA Technical Paper 3684. Johnson Space Center, Houston TX. NASA Tech Brief, (1997).

Gravity and Bone Growth

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Funding:

UPN/Project Identification: 199-26-12-36

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$89,955

Responsible NASA Center: Ames Research Center

Task Description:

The original rationale for this grant was the following. Cyclic, mechanical loading is clearly a major determinant of bone volume and bone strength. However, the molecular mechanisms involved in translating the mechanical signal to a cellular response are not well-defined and have only been amenable to investigation with the advance of molecular techniques. Mechanical unloading of the skeleton results in decreased bone mass and physical strength. However, within 24 hr of reloading the cortical periosteal bone after 9d of hindlimb unloading, >250% increase in message expression for certain bone marker proteins occurred; the response was less dramatic in cancellous bone. The response in cortical bone cells suggests that our model can be tested in these cells using hindlimb unloading. The response in cancellous bone cells suggests that testing the model in this cell type may produce less conclusive results than cortical bone.

During the third grant year, hindlimb studies were conducted that completed the timing sequence for the response to reloading following unloading so that a paper could be completed for submission to a peer-reviewed journal. The data in the paper will compare the data from a middeck experiment with data from the rat model. The data from the hindlimb studies will also show that cancellous bone responds more rapidly to reloading than does cortical bone; the postflight time sequencing missed the cancellous response time. The centrifuge experiments completed last year were surprising as the data from these short duration studies in rats suggested, if anything, a decreased rather than increased loading during centrifugation. However, these surprising data led us to investigate methods of detecting locomotion biomechanical loads during exposure to altered gravity to determine if locomotion and biomechanical loads are altered during increased gravitational loading.

Hindlimb unloading time-course studies focusing on clarifying the response in cancellous bone between 2 and 12 hours following a 9d suspension completed the time sequence for reloading events in bone. The studies tested the hypothesis that the sequence downstream of initial signaling events includes activation of the rapidly responding nuclear proto-oncogenes associated with proliferation and differentiation (*i.e.*, *c-myc*, *c-jun*, or *c-fos*), followed by increased expression of late responding genes associated with matrix production and maturation (*i.e.*, transforming growth factor- β [TGF- β], type I precollagen [ColI], and alkaline phosphatase [AlkP]). Subsequent to matrix maturation, activation of genes associated with matrix mineralization (*i.e.*, collagenase and osteocalcin) occurs. These events culminate in formation of new bone which can be detected as an increase in tissue and serum levels of corresponding matrix proteins as well as increased bone mineralization rates, bone

mineral content, and bone mass and accurately describes how mechanical loading stimulates bone formation. Multiple experiments to complete the necessary data to establish the reloading sequence of molecular events focused on the response of cancellous and cortical bone marker proteins and growth factors during the first 24hr of reloading after a 9d unloading period. We found that cancellous bone does respond to reloading and, in fact, responds more rapidly than cortical bone. We found suggestions that the initial stimulus is most likely TGF β , followed by elevation of mRNA of proteins indicative of matrix formation (ColII), and then by indicators of bone mineralization (OC). Our initial conclusions from the flight suggested that cancellous bone was less responsive to reloading than cortical bone. However, the flight data were obtained 4 hr, 24 hr, and 72 hr following flight; data from the hindlimb unloading experiments showed that cancellous bone responds similarly to cortical bone, but the response in cancellous bone is complete within 24 hr whereas the cortical bone response begins around 24 hr after reloading. A paper comparing these data with those obtained from flight has been submitted for review.

The centrifuge experiments completed last year were surprising as the data from these short duration studies in rats suggested, if anything, a decreased rather than increased loading during centrifugation. However, these surprising data led us to investigate methods of detecting locomotion biomechanical loads during exposure to altered gravity to determine if locomotion and biomechanical loads are altered during increased gravitational loading. We collaborated with Dr. John Szivek (University of Arizona) to make these measurements. Dr. Szivek's laboratory has extensive experience with the mounting of strain gauges in rats and accurately measuring strain *in vivo* in rats for up to 12 weeks. Subminiature strain gauges CEA-06-015uw-120 (Measurements Group Inc., Raleigh, North Carolina) were wired for rat femurs using shielded cable. A bead of polymethylmethacrylate was placed over the surface of the gauge wire junction and coated with nitrile rubber. A layer of air drying acrylic followed by a layer of polyurethane was placed on the gauges. The gauges were then sterilized with ethylene oxide and mounted on the femur. Animals were anesthetized, an incision was made on the left leg and the femur exposed. The bone was gently scraped to remove the periosteum and the gauge is glued to the femur using a cyanoacrylate adhesive. Wires from the gauges were connected to a series of signal conditioners and a multichannel chart recorder. The incision was closed and the animal is allowed to recover. We found that while walking, the change in strain approximated 80 μ strain with changes in peak values of 500 μ strain when pivoting. In the relaxed state during hindlimb elevation, changes of 200 μ strain were recorded. When the hindlegs were extended, strain changes increased to 390 μ strain. Floating and swimming produced changes in strain of -180 to +20 μ strain. These data clearly indicate the feasibility of measuring strains *in vivo* in rats of different ages during altered skeletal loading, as well as during normal ambulation. We hope to use these techniques to study rats during centrifugation. In conversations with Dr. Nancy Daunton, we learned that her locomotion studies of centrifuged rats suggest that locomotion is altered with increased gravity and that the gait is shortened and the limb lift decreases with increased load. This altered locomotion might also explain the changes in biomechanical skeletal loading with increased gravity.

The musculoskeletal system is adapted to the cumulative influence of forces generated by muscles and body weight which are imposed on bone during normal daily activity on Earth. The bone tissue architecture reflects both the past and current loading history as well as metabolic and genetic influences. The levels of force and patterns of loading differ greatly in different regions of the skeleton and among individuals. When the typical patterns of loading are altered by space flight, immobilization, or exercise, the rate and magnitude of skeletal adaptation varies according to the change in skeletal loading and intrinsic factors.

Cyclic, mechanical loading is clearly a major determinant of bone volume and bone strength. However, the molecular mechanisms involved in translating the mechanical response to a cellular response are not well-defined and have only been amenable to investigation with the advance of molecular techniques. Mechanical unloading of the skeleton results in decreased bone mass and physical strength. However, reloading the skeleton after 9d of mechanical unloading in young rats suggests that greater than a 300% increase in message for certain bone marker proteins occurs within 24 hr. The remarkable increase in message production suggests that upstream molecular events associated with bone formation possibly may be mapped out using the rat suspension model of unloading the hindquarters. The detailed investigations of *in vitro* molecular events and bone markers provide the starting points and timing for these *in vivo* studies. The significance of these studies will be an extension of

our understanding of the basic mechanisms associated with activation of bone formation *in vivo* and the sequence of molecular events following different loading regimes. If the hypothesis that increased mechanical loading stimulates osteoblast cells with activation of specific oncogenes (e.g., *c-myc*, *c-jun*, or *c-fos*) that, in turn, increases the message and tissue levels of specific bone markers (i.e., TGF- β , collagen type I, osteocalcin) leading to increased production, maturation, and mineralization of the organic matrix is valid, then it should be possible to bypass the mechanical signal in an unweighted bone or skeleton by regulating the levels of the signaling molecules normally induced by loading. Thus, the proposed research has implications beyond the immediate scope of this proposal.

FY97 Publications, Presentations, and Other Accomplishments:

Bikle, D.D., Halloran, B.P., and Morey-Holton, E. "Space flight and the skeleton: Lessons for the earthbound." *Endocrinologist*, 7, 10-22 (1997).

Brommage, T.G., Smolyar, I., Doty, S.B., McMahon, J.M., and Holton, E. "Circadian growth rate variability of lamellar bone in rats exposed to microgravity." *ASGSB Bull.* 11(1), 39 (1997).

Dehority, W., Halloran, B., Bikle, D., Harris, J., Curren, T., Kostenuik, P., and Morey-Holton, E. "Bone, muscle, and hormonal changes induced by hindlimb elevation in the mature rat." *J. Bone Min. Res.*, 12 (Suppl. 1), S466 (1997).

Dehority, W., Halloran, B., Bikle, D., Harris, J., Curren, T., Kostenuik, P., and Morey-Holton, E. "Bone, muscle, and hormonal changes induced by hindlimb elevation in the mature rat." *ASGSB Bull.*, 11(1), 52 (1997).

Evans, G.L., Morey-Holton, E., and Turner, R.T. "Spaceflight has compartment and gene specific effects on mRNA levels for bone matrix proteins in rat femur." *J. Appl. Physiol.* (In Press).

Halloran, B.P., Bikle, D.D., Harris, J., Tanner, S., Curren, T., and Morey-Holton, E. "Regional responsiveness of the tibia to intermittent administration of parathyroid hormone as affected by skeletal unloading." *J. Bone Min. Res.*, 12, 1068-1074 (1997).

Holmuhamedov, E.L., Moursi, A., Lull, J., Morey-Holton, E., Damsky, C., and Globus, R.K. "Laminin regulates differentiation and survival of cultured osteoblasts." 6th International Congress on Cell Bio. & 36th Am. Soc. for Cell Bio. Ann. Mtg. Special Poster Session, H113 (December 11, 1996).

Kostenuik, P., Halloran, B., Morey-Holton, E., Harris, J., and Bikle, D. "Skeletal unloading alters the osteoblast phenotype *in vitro* and *in vivo*." *J. Bone Min. Res.*, 12 (Suppl. 1), S283 (1997).

Kostenuik, P.J., Halloran, B.P., Morey-Holton, E., and Bikle, D.D. "Osteoprogenitor cell proliferation and differentiation is inhibited by skeletal unloading in the rat." *ASGSB Bull.*, 11(1), 51 (1997).

Kostenuik, P.J., Halloran, B.P., Morey-Holton, E., and Bikle, D.D. "Skeletal unloading alters the *in vitro* differentiation of rat osteoprogenitor cells." *Am. J. Physiol.* (In Press).

Morey-Holton, E.R. and Arnaud, S.B. "Bone and calcium metabolism: The importance of gravitational loading and diet." *Japanese Society of Space, Aviation, and Environmental Medicine*, Tsukuba, pp. 1-6 (November 15, 1996).

Morey-Holton, E.R., and Globus, R.K. "Hindlimb unloading of growing rats: A model for predicting skeletal changes during spaceflight." *Bone* (In Press).

Effect of Gravity on the Regulation of Circadian Rhythms

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Students Funded Under Research: 5

Post-Doctoral Associates: 0

FY 1997 Funding: \$118,910

Task Description:

Earth organisms have evolved in an environment with a static gravitational force and daily environmental cycles such as light, temperature, and humidity. Consequently, an organism's physiological variables exhibit rhythmicity with a near 24 hour period (circadian rhythms). Alterations in the gravitational field affect rhythmicity, but it is not yet known if a change in gravity affects rhythmic functioning by acting directly upon the suprachiasmatic nucleus (SCN), which is the central neural pacemaker. These experiments will examine both the effect of a hypergravity on circadian function and the neural mechanism through which this action takes place. This will be accomplished by testing the hypotheses that exposure to 2-G will depress both circadian rhythms and gene activity within the SCN and that recovery of rhythmicity will be correlated with recovery of gene activity in the pacemaker. Further, if 2-G does act as a synchronizer for circadian rhythms, it will also entrain the expression of protein synthesis within the SCN.

During this funding period we have made significant progress in accomplishing the proposed specific aims from the original grant.

HYPOTHESIS 1: Following 21 days of chronic 2-G exposure, experimental rats exhibited a return of circadian rhythms and normal c-Fos reactivity within the SCN relative to 1-G control groups.

METHODS. Experimental rats (n = 10) were placed on an 18 foot diameter centrifuge and exposed to 21 days of 2-G via centrifugation; one group (n = 5) was exposed to a one hour light pulse (fluorescent light, 300 lux) from 21:00-22:00 (CT 13-14), while the second group (n = 5) was not exposed to light. Following the light pulse, both experimental groups were then immediately returned to 1-G and sacrificed for histology.

RESULTS. There was a significant difference in the number of neurons reactive for c-Fos protein between the light pulse and no light pulse groups.

CONCLUSION. The results of this study demonstrate that the circadian rhythms and the photic induction of c-Fos protein expression recovers to normal after 21 days of 2-G exposure via centrifugation. The deficits in SCN c-Fos immunoreactivity immediately following 2-G and recovery following 21 days of continuous 2-G suggests that the pacemaker function of the SCN is directly affected.

HYPOTHESIS 2: Exposure to a one hour 2-G pulse will phase shift the circadian rhythms of body temperature and activity.

METHODS. Experimental rats were exposed to one hour 2-G pulse via centrifugation at either CT 4, 10, 16, or 22. Following 2-G exposure the rats were placed into constant dark conditions in order to measure any potential phase shifts in the circadian rhythms of body temperature and activity.

RESULTS. Exposure to a one hour 2-G pulse causes a significant phase shift in the circadian rhythms. When examined over the 24 hour period, 2-G pulses induce a consistent phase advance and no circadian delays.

CONCLUSION. A phase shift in circadian rhythms indicates that the circadian pacemaker has been affected. This would suggest that the one hour 2-G pulse may directly affect the pacemaker by acting as a time cue. This is consistent with the effect of a one hour 2-G pulse on c-Fos protein expression within the SCN. Exposure to 2-G resulted in a decrease in c-Fos reactivity at all times examined. There were never an increase in c-Fos protein expression by 2-G exposure. This suggests a relationship between phase advances and a decrease in c-Fos protein in the SCN. Further studies are needed to determine if 2-G exposure can act as a circadian Zeitgeber that can entrain circadian rhythms.

SUMMARY OF TASK PROGRESS REPORT

Our studies have demonstrated the effect of hypergravic fields on circadian rhythms. These studies demonstrated that exposure to hypergravic fields affects the neural mechanism of gene expression within the SCN. These insights may be critical for understanding the process of adaptation to the space environment. In addition, the rhythm experiments demonstrate how 2-G exposure depresses circadian rhythm amplitude and potentially acts as a circadian timing cue.

Space flight has taken humans and animals into a new environment, removed from Earth's normal gravitational field and daily cyclic fluctuations. These environmental changes induce an adaptive response in many physiological systems that may temporarily or permanently result in dysfunction. For example, Apollo astronauts experienced perceptions of cold discomfort, even though body and ambient temperatures remained in the normal range. Whether the perception of cold discomfort was due to gravitational effects on thermoregulatory mechanisms or possible desynchrony of temperature rhythmicity induced by abnormal circadian rhythms is not known. Another example is that of space adaptation syndrome which is primarily thought to involve microgravity's effect on vestibular and kinesthetic sensory systems. Further, desynchronization of circadian rhythms during space flight may contribute to this adaptation and result in physiological discomfort analogous to jet-lag. Surveys reveal that most crew members suffered from sleep disruption during the missions, while cosmonauts on long-term missions appear to have been particularly vulnerable to the effects of fatigue. It is thus not surprising that some astronauts use sleeping pills. Misalignment of circadian rhythms may play a prominent role in these disturbances. These few examples demonstrate that the biomedical problems of space will require an examination of the respective contribution of gravity and circadian rhythmicity to these adaptation syndromes. Chronic acceleration via centrifugation may be a useful ground-based research tool in which to examine the relationship between gravity and the circadian timing system. In addition, understanding the process of adaptation by the circadian timing system to altered gravitational fields may also provide useful insights into Earth related deficits in circadian rhythms, such as sleep disorders, jet-lag, and shift work.

FY97 Publications, Presentations, and Other Accomplishments:

Murakami, D.M. and Fuller, C.A. "The effect of chronic 2G exposure on retino-hypothalamic tract function." ASGSB Bull. (1996).

Fully Implantable Integrated Silicon Biotelemetry

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Funding:

UPN/Project Identification: 199-80-07-03
Initial Funding Date: 1995
Students Funded Under Research: 1
FY 1997 Funding: \$247,516

Solicitation: 93-OLMSA-07
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Post-Doctoral Associates: 1

Task Description:

The primary objective of this project is the development of miniature, fully implantable multichannel biotelemetry systems for the measurement of physiological parameters. These low-power systems will transmit recorded information using an implanted transmitter, and are targeted to measure biopotentials, blood pressure, core body temperature, multi-axis acceleration, and pH in small unrestrained rodents and primates. These devices will eventually be used by NASA in its animal studies for both ground-based and space experiments.

Although the general direction of our research efforts still remains the development of a complete system, upon discussion with researchers at NASA Ames research center in August 1996, our efforts have been focused on two areas that are of critical need to NASA, as discussed below.

1) Long-term baseline stability is a major concern in implantable pressure transducers. Baseline drift in these sensors impede their use in chronic applications unless frequent calibrations are performed. These calibrations are time consuming and impractical when access to the animal is not possible. To overcome the shortcomings of current devices, a new approach of designing a miniature pressure sensor array using silicon micromachining technologies to account and compensate for any long-term drift has been taken and is currently under development. This will have a great impact not only in biotelemetry measurements in animals but also in many implantable medical devices that require chronic long-term pressure measurements. In addition to pressure sensors, this project also aims at the development and implantation of sensors for the measurement of multi-axis acceleration and neural biopotentials in unrestrained animals.

2) We will design and fabricate low-power, high-performance data acquisition and telemetry circuits to be used with various sensors in implantable telemetry units. These will include: biopotential amplifiers, switched-capacitor charge readout circuits for capacitive sensors, interface circuits for resistive sensors, low-power multiplexer and A/D converters, microcontroller, and bi-directional telemetry circuitry. These circuits can be fabricated through commercial foundries and will be available to various investigators. The development of standard circuit blocks that can be easily retrieved and used in implementing new biotelemetry systems will significantly reduce the development and fabrication time. These circuit blocks will be available to other potential users in the future, and it is the goal of this project to develop a library of these circuit blocks.

During the second year of this project, we concentrated our efforts in two major areas. First, the design and fabrication of a capacitive pressure sensor array for extra-vascular blood pressure measurement. And second, the design and fabrication of a low-power A/D converter and a capacitive interface chip. In this section, we will briefly describe the results of our work in these two areas.

As mentioned in the abstract, stable low baseline drift pressure transducers are in high demand. This is especially true for implantable systems where frequent calibrations are not always possible or convenient. It is believed that capacitive pressure sensors show a lower drift compared to their piezoresistive counterpart. This is due to the global effect of pressure on the diaphragm in capacitive transducers compared to the local pressure summing points in piezoresistive devices. Therefore, any package stress (the main source of drift) is averaged out in a capacitive device and causes less drift and long-term stability problem. During the last year, we have designed and fabricated a capacitive pressure sensor array using silicon bulk micromachining technology for extra-vascular (tonometric) blood pressure measurement. This device consists of three pressure sensors located in series which contact the blood vessel from the outside and are flush to the vessel wall. A custom-made titanium cuff houses the sensor substrate and interface read-out chip. The titanium cuff is fastened using four small screws clamping the blood vessel against the sensors. The interface chip contains a switched-capacitor read out circuitry, a low-power A/D converter, and a control logic. The control logic allows the user to sample any of the three sensors and average the output. We are currently testing the sensors and the interface chip *in vitro* and are planning to implant the system in animals in the coming year.

The second major area of our effort during the second year was the design and fabrication of different circuit blocks required for various biotelemetry applications. We concentrated our efforts on the analog signal processing and wireless telemetry parts such as interface electronics, A/D conversion, and low-power transceivers. We have designed and fabricated (through MOSIS) a successive approximation A/D converter and a capacitive readout circuit for pressure and acceleration sensors. These circuits have been tested and have shown full functionality. The A/D converter has 8-bits of resolution and consumes 100 μW power using a 3 V supply (sampling at 3 ksample/sec). The switched capacitor interface chip consumes 600 μW and has a resolution of 1 fF (noise limit of < 2 mV). We have successfully used the switched capacitor read-out to test our pressure transducers. In addition, we have also designed and fabricated a low-power low-loss silicon platform for bi-directional telemetry. Using this platform, we were able to fabricate high quality factor inductors and capacitors. These components are needed in order to implement low-power transceivers. We have used this platform to design a low-power Colpitts transmitter. The transmitter operates from a 3V battery and consumes 200 μA when operated continuously and 100 μA when amplitude modulated (on-off keying) at a rate of 1 Mbps. The transmitter has an area of $5 \times 5 \text{ mm}^2$ and a range of ~ 3 feet.

In the coming year, we are planning to finish the design and fabrication of the remaining circuit blocks. These include a low-power biopotential amplifier, interface circuit for resistive sensors, microcontroller, and receiver. In addition, we will test and fully characterize the blood pressure sensor array and perform *in vivo* tests. Meanwhile, we will be closely collaborating with our NASA colleagues at Ames Research Center and are providing them with the fabricated interface chips and pressure transducers.

The main goal of this project is to develop miniature implantable telemetry microsystems for recording a variety of physiological parameters from unrestrained rodents and primates. This multichannel system will enable scientists to develop much better understanding of basic physiological and biological processes as the body undergoes various changes both on Earth and eventually in space under weightlessness. Current systems are too bulky and limited and do not allow the collection of this information reliably over extended periods of time. The system being developed in this research is also the first system which will allow the recording of high-bandwidth, low-amplitude action potentials generated by neurons. Although systems like this can be extensively used in space applications for monitoring the health of astronauts, they are also immediately and directly useful in monitoring patient health under various conditions. Miniature implantable measurement systems can allow the internal health signs of a patient to be monitored either during surgery or in normal daily life. This will improve the reliability of measurement and will enhance the quality of care being delivered, and can eventually reduce health care costs.

FY97 Publications, Presentations, and Other Accomplishments:

Ziaie, B., Kocaman, N., and Najafi, K. "A generic micromachined silicon platform for low-power, low-loss miniature transceivers." Proceedings, 9th. Int. Conf. on Solid State Sensors & Actuators, pp. 257-260, Chicago (June, 1997).

Mechanisms of Sensorimotor Adaptation to Centrifugation

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$200,000

Responsible NASA Center: Johnson Space Center

Task Description:

It is generally agreed that changes in gravitational tilt information are of particular importance to the recoding of sensorimotor and perceptual responses during adaptation to space flight and readaptation to Earth. The basic premise of our investigations is that gravity-equivalent centripetal acceleration induced by centrifugation can be used as an inflight sensorimotor countermeasure to retain and/or promote quicker recovery of crew members' ability to detect and respond appropriately to different gravito-inertial conditions. The goal of our research is to investigate the physiological changes elicited by centrifugation to characterize its use in providing an artificial gravity environment. We propose to use a ground-based, short-radius (one meter) centrifuge to study mechanisms of adaptation to an altered gravity environment (sustained tilt) as an analog to sensorimotor adaptation to space flight. These experiments will build a foundation for future flight studies to assess the mechanisms of spatial orientation function and plasticity during extended exposures to microgravity provided by the U.S. and/or Russian Mir Space Stations.

The first two years of this grant have resulted in the development of a short-arm centrifuge facility, and has provided insightful findings on the effects of sustained roll-tilt on the spatial coding of eye movements, optokinetic nystagmus, and ocular counterrolling, as well as on comparisons of static and dynamic roll-tilt responses during centrifugation at different radii. In particular, we have observed during roll-tilt stimuli that: (1) spatially directed eye movements are more accurate when made relative to the perceived gravito-inertial reference frame versus a head reference frame; (2) there is a consistent reduction in the magnitude of horizontal optokinetic nystagmus, without systematic cross-coupling effects; and (3) there is instability (or drift) associated with conjugate torsion. This grant has also supported eccentric vestibulo-ocular reflex studies with subjects in a tangential orientation. A set of companion experiments using longer-radius centrifugation (2- 6 m) at the Naval Aerospace Medical Research Laboratory in Pensacola, Florida will allow us to evaluate differences between short

(< 1 m) and long-radius centrifugation for future space applications with operational constraints which will limit the size of inflight centrifuge facilities. In addition, this grant has supported further development of sensorimotor models which have been used to predict three dimensional VOR responses during complex motion stimulation involving hypergravity.

With this background, we are now ready during this final year to examine sensorimotor adaptation to centrifugation using different combinations of voluntary movements and visual references. Sustained centrifugation results in a hypergravity tilt stimulus along the resultant of the centrifugal and gravitational force vectors. Our approach will be to utilize pre- and post-centrifugation comparisons to evaluate adaptation to a sustained hypergravity tilt. We will assess adaptive changes in the spatial coding of the gaze and VOR using videography, and assess adaptive changes in vestibulo-spinal function using computerized dynamic posturography.

Our research is specifically directed toward the use of centripetal acceleration as a gravity-equivalent sensorimotor countermeasure to promote dual adaptation to orbital and Earth gravitoinertial environments. Although there are currently no established test methods for assessing otolith function in a clinical setting, canal-otolith interaction during eccentric rotation has been used by several investigators as a basis for assessing otolith function. Our research will provide further insight into the normal processing of graviceptor input and will provide new information on the dynamics of spatial orientation adaptation with discordant sensory input. We believe that this research is relevant to both basic and applied clinical questions related to mechanisms of vestibular processing of gravitoinertial stimuli. New understanding gained in our research on mechanisms of vestibular system conditioning will be fundamental to further development of both future space flight countermeasures and potentially new vestibular rehabilitation techniques.

FY97 Publications, Presentations, and Other Accomplishments:

Glasauer, S. and Merfeld, D.M. "Modelling three dimensional vestibular responses during complex motion stimulation" in "Three-Dimensional Kinematics of Eye, Head, and Limb Movements." Edited by: Fetter, M., Misslisch, H., Tweed, D., and Halswanter, T. Harwood Academic Publishers GMBH, Amsterdam (In Press).

Merfeld, D.M. "Must all action halt during sensorimotor mismatch?" *Behav. & Brain Sci.* (In Press).

Wood, S.J., Paloski, W.H., and Reschke, M.F. "Spatial coding of eye movements relative to perceived head and earth orientations during static roll-tilt." *Exp. Brain Res.* (In Press).

Perceived Self-Motion Assessed by Computer-Made Animations

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Funding:

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Students Funded Under Research: 4
FY 1997 Funding: \$85,803

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Post-Doctoral Associates: 0

Task Description:

Neurosensory adaptation to microgravity and readaptation to Earth-normal gravity has been assessed by recording astronauts' perceptual, eye-movement, and postural-control responses. Recent research indicates that time-courses of adaptation and readaptation for these three response classes differ. This suggests that complete understanding of adaptation/readaptation processes requires refined analysis of perceptual responses. Our overall goal is development of procedures to enhance assessment of spatial orientation, specifically self-orientation and self-motion perception. Our specific objective is to develop and evaluate computer-generated animations as potential tools for measuring perception. The proposed research will compare perceived self-motion and self-orientation reports obtained using animations with those obtained using verbal reports. Subjects will be exposed to two classes of motion stimuli: 1) pitch oscillation combined with visual scene translation with respect to the subject in the Tilt-Translation Device (TTD) Preflight Adaptation Trainer, and 2) cross-coupled angular acceleration on a rotator designed to elicit complex perceived self-motion. Self-motion perception will be assessed by 1) selection by the subject of animations from a stored library of animations, 2) selection by the subject of verbal reports from a stored library of reports, 3) concurrent subject-generated verbal reports, and 4) generation of animations by the subjects in "real time." The hypothesis that more reliable, sensitive, and interpretable data will be obtained from the animation selection procedures than from verbal report procedures will be evaluated. The proposed research is intended to enhance understanding of adaptation to microgravity and readaptation to Earth-normal gravity and, in turn, to facilitate development of countermeasures for neurosensory disturbances during adaptation and readaptation.

INTRODUCTION

Our overall goal is the development of procedures to enhance assessment of spatial orientation, specifically self-orientation and self-motion perception. Our specific objective is to develop and evaluate computer-generated animations as potential tools for measuring perception. We compared perceived self-motion reports obtained using animations with those obtained using verbal reports.

METHODS

36 subjects reported perceived self-motion following exposure to complex inertial-visual motion stimuli. Twelve subjects were assigned to each of 3 perceptual reporting procedures: animation movie selection (AMS),

verbal report selection (VRS), and verbal report generation (VRG). The question addressed was: do reports produced by these procedures differ with respect to complexity and reliability? Following repeated (within-day and across-day) exposures to 4 different "motion profiles" (see Appendix), subjects in the AMS group selected, from a set of movies presented on a laptop computer, the movie that corresponded most closely with their motion experience. Subjects in the VRS group selected from a set of verbal description presented in a booklet, and VRG subjects provided their own self-motion verbal descriptions. "Complexity" and reliability "scores" were calculated.

RESULTS

Mean (and standard error of the mean) within-day reliability, across-day reliability, and complexity scores for the data are presented in Table 1.

TABLE 1.

MEAN (SEM) COMPLEXITY AND RELIABILITY SCORES FOR 3 REPORTING PROCEDURES

	COMPLEXITY	WITHIN-DAY	ACROSS-DAY
AMS	0.546 (0.055)	0.319 (0.075)	0.228 (0.071)
VRS	0.577 (0.051)	0.302 (0.071)	0.221 (0.063)
VRG	0.431 (0.055)	0.327 (0.069)	0.295 (0.078)

The means were essentially equivalent for movie selection and verbal report selection procedures: no statistically significant differences between reporting procedures were observed. The data suggest that reports by verbal report generation subjects were less complex than for the other conditions. The hypothesis that movie selection would be more reliable than the verbal report procedures was not supported.

Frequency of "hill" and "valley" descriptions for 2 motion profiles for each of the 3 reporting procedures are presented in Table 2.

TABLE 2.

NUMBER OF SELECTIONS BY PROFILE AND REPORT TYPE

	PROFILE H8				PROFILE S8			
	AMS	VRS	VRG	SUM	AMS	VRS	VRG	SUM
SUM								
HILL DESCRIPTION	8	13	16	37	0	0	3	3
VALLEY DESCRIPTION	0	0	0	0	24	22	14	60

Clearly, the H8 and S8 profiles elicited different responses (collapsed across reporting procedures, $X^2 = 85.7$; $p < 0.0001$).

DISCUSSION AND CONCLUSIONS

There are several possible reasons for the failure of this experiment to demonstrate clearly expected advantages of animations. First, appropriate, careful training to use a standard self-motion description vocabulary may eliminate possible differences between reporting procedures. Subjects may be better able to describe motion verbally than is usually believed. Second, the motions may not have been sufficiently complex. Based on the stimulus motions, only fairly simple self-motions (2 degrees-of-freedom (DOF) translational and 1 DOF rotational) would be expected. Third, movies/verbal descriptions depicting combined scene and self-motion perception, which almost certainly corresponded with the subjects' actual experience, were not used. Fourth, individuals probably differ with respect to how they represent motion cognitively - some may use pictorial representations, whereas others may use verbal descriptions. Finally, possible differences in self-motion experiences for VRG subjects were not easily assessed because descriptions were often relatively simple. For example, many subject-generated verbal reports were vague at best, and omitted important information (e.g., whether a "ramp back" had an upward or downward slope). For AMS and VRS subjects, selections were made

from a set of precise movies / reports; consequently, it was more likely that even subtle differences in the self-motion experience elicited by repeated exposures to a particular profile would result in selection of different movies / reports.

The hypothesis that a "real-time" animation reporting procedure, which permits omission of motion vocabulary training, will produce more reliable data than verbal reports is being examined currently. Because subjects cannot readily use the animation movies selection procedure without training, Experiment 2 employs cross-coupled rotation stimuli and a new reporting procedure: animation generation. This is accomplished by having the subject manipulate a mannequin so that the mannequin's motion corresponds to the perceived self-motion. Polhemus Fastrak sensors embedded in the mannequin permit "real-time" representation of the motion on a monitor as well as recording of that motion for later analysis.

Although not the primary purpose of this research, the results indicate that different combinations of tilt with respect to gravity and translation of a visual surround with respect to the subject can yield consistently different patterns of self-motion trajectory. The hypothesis that neural signals representing visual surround velocity are additive with those representing pitch position was supported (see Harm et al., *Aviat. Space Environ. Med.* 1993, 64, 820-26).

APPENDIX

MOTION DESCRIPTIONS

Hill description. Simultaneous pitch and hill: pitch forward as translate forward over the hill; pitch rearward as translate rearward back over the hill.

Valley description. Simultaneous pitch and valley: pitch rearward as translate forward down into and up out of a valley; pitch forward as translate rearward back down into and up out of the valley.

MOTION STIMULI

Motion stimuli were produced by the Tilt Translation Device, a 1 DOF moving base that combines pitch motion of the subject with translation of a visual surround with respect to that subject. Relationships between subject and visual surround motions can be easily manipulated. When both are sinusoidal, the visual surround may move either toward or away from the subject as they pitch forward; i.e., the phase angle between visual surround and subject motions can be controlled (see Harm et al., 1993).

TABLE 1. MOTION PROFILES

NAME	MFP	MRP	PHASE	FREQ. - Hz
H8	10	13	20	0.08
S8	5	9	180	0.08

MFP: maximum forward pitch (deg).

MRP: maximum rearward pitch (deg).

PHASE: amount by which maximum forward surround position (farthest rearward subject position in the surround) leads maximum forward pitch of the surround and subject (deg).

For profile H8, peak velocity of visual-surround-induced forward self-translation occurs during the transition from maximum forward to maximum rearward pitch; and, peak visual-surround-induced rearward self-translation velocity occurs during the transition from maximum rearward to maximum forward pitch.

For profile S8, peak visual-surround-induced forward self-translation velocity occurs during the transition from maximum rearward to maximum forward pitch; and, peak visual-surround-induced rearward self-translation velocity occurs during the transition from maximum forward to maximum rearward pitch.

The procedures developed in this research should enhance assessment of otolaryngology clinic patients who suffer from equilibrium system disturbance. The costs, both personal and financial, of falling and other accidents

related to disequilibrium, are enormous. Consequently, research to refine assessment of vestibular function has been given a high priority by the National Institute of Deafness and Communicative Disorders.

Spatial orientation perception is extraordinarily difficult to study with otolaryngology clinic patients. This proposal derives from the postulate that non-verbal perceptual reporting procedures using animations may be valuable for spatial orientation assessment. Possible advantages of animations include: 1) they require only limited verbal communication and can readily be used with children, people who do not speak fluently the language of the physician, elderly patients, etc.; 2) they permit illustration of complex combinations of motion such as simultaneous translation and rotation; 3) they permit illustration of independent motion of body components such as head pitch combined with torso yaw; and 4) they permit illustrating separation of visual scene motion from self-motion.

The procedures developed through this research are included in a proposal to study spatial cognition in normal subjects and patients who suffer from equilibrium system disorders. We anticipate that results from several cognitive/perceptual test procedures, including animation generation, as well as those from rotational video oculography and the Equitest Sensory Organization tests will permit more precise identification of underlying deficits than has been possible previously.

FY97 Publications, Presentations, and Other Accomplishments:

Benson, A.J., Guedry, F.E., Parker, D.E., and Reschke, M.F. "Microgravity vestibular investigations: Perception of self-orientation and self-motion." *J. Vestib. Res.* (In Press).

Harm, D.L., Parker, D.E., Reschke, M.F., and Skinner, N.C. "Relationship between selected orientation rest frame, circular vection and space motion sickness." *Proceeding of International Workshop on Motion Sickness, Marbella, Spain (May, 1997).*

Parker, D.E. "The relative roles of otolith organs and semicircular canals in producing space motion sickness." *J. Vestib. Res.* (In Press).

Parker, D.E., Harm, D.L., Sandoz, G.R., and Skinner, N.C. "Self-motion perception: Assessment by computer-generated animations." *12th Man in Space Symposium: The Future of Humans in Space, Washington, DC, pp. 31-2 (June, 1997).*

Prothero, J.D., Draper, M.H., Furness, T.A., Parker, D.E., and Wells, M.J. "The use of an independent visual background to reduce simulator side-effects." *Proceeding of International Workshop on Motion Sickness, Marbella, Spain (May, 1997).*

Facilitated Blood Pressure Control by Skin Cooling: Autonomic Mechanisms

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 Students Funded Under Research: 2
 FY 1997 Funding: \$0

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 Expiration: 1999
 Post-Doctoral Associates: 0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The overall objective of this proposal is to assess the efficacy of whole body skin cooling as an operationally relevant countermeasure to activate the sympathetic nervous system and improve orthostatic tolerance in hypovolemic people. Three questions will be addressed using these experiments: 1) How is the sympathetic nervous system activated by cooling of skin? 2) Is there a dose-response relationship between the amount of surface area cooled, the extent to which skin is cooled, and resultant changes in autonomic control of blood pressure? 3) Does an optimal strategy of body surface cooling preserve cardiovascular regulation following acute hypovolemia?

Funding of this work has been temporarily suspended pending Dr. Pawelczyk's completion of his responsibilities as a primary Payload Specialist for STS-90 (NeuroLab). Carryover of 1995 funding permitted several major projects to be completed.

The focus of our current research has been to develop and understand a non-invasive model to simulate hypovolemia similar to that which might be encountered after space flight. We have focused on the use of passive heating using water-perfused suits to clamp skin temperature and minimize evaporative cooling of skin. This causes a substantial redistribution of blood from thoracic, abdominal, and splanchnic compartments to skin, which produces a central response that mimics hemorrhage or hypovolemia.

Because aging may emulate a condition of less effective cardiovascular regulation, like space flight, we passively heated seven young (Y; 23 ± 1 yr) and seven older (O; 70 ± 3 yr) men to their individual limit of thermal tolerance. Measurements included heart rate (HR), Q_c (acetylene rebreathing), central venous pressure (peripherally inserted central catheter), blood pressures (brachial auscultation), skin blood flow (from increases in forearm blood flow by venous occlusion plethysmography), splanchnic blood flow (SpBF, indocyanine green clearance), renal blood flow (RBF, *para*-amino-hippurate clearance), esophageal (T_{es}), and mean skin temperatures (T_{sk}). Q_c was significantly lower in the O compared to the Y men (Y: 11.1 ± 0.7 vs. O: 7.4 ± 0.2 L \cdot min $^{-1}$, at the limit of thermal tolerance; $p < 0.05$) despite similar increases in T_{es} , T_{sk} , and time to reach the limit of thermal tolerance. A lower SV (Y: 99 ± 7 vs. O: 68 ± 4 ml \cdot beat $^{-1}$; $p < 0.05$), most likely due to an attenuated

increase in inotropic function during heating, was the primary factor for the lower Q_c observed in the O men. The Y and O men had similar increases in HR; however, when expressed as a percent of maximal HR (%HRmax), the O men relied on a greater proportion of their chronotropic reserve to obtain the same HR response (Y: $62 \pm 3\%$ vs. O: $75 \pm 4\%$ HRmax; $p < 0.05$). Furthermore, the O men redistributed less blood flow from the combined splanchnic and renal circulations at the limit of thermal tolerance (Y: 960 ± 80 vs. O: $720 \pm 100 \text{ ml} \cdot \text{min}^{-1}$; $p < 0.05$). As a result of these combined attenuated responses, the O men had a significantly lower increase in total blood flow directed to the skin.

Since cardiac preload was decreased during passive heat stress (assuming that CVP is an adequate index of ventricular filling under these experimental conditions), the increase in Q_c cannot not explained by the Frank-Starling mechanism of the heart, particularly under these conditions where muscle pumping was not active. Rather, these results suggest that the ability of older individuals to increase Q_c in these conditions is limited by diminished beta-adrenergic stimulated increases in cardiac inotropism.

In a companion investigation, the same subjects underwent a period of 20 minutes of 60 degree, foot-supported head-up tilting before and after a period of passive heating to produce central hypovolemia. In response to tilting in the thermoneutral condition splanchnic vascular resistance was greater in the older men (Y: 90 ± 4 vs. O: 119 ± 14 units; $p < 0.05$), but the young men increased forearm vascular resistance (FVR; Y: 32.4 ± 4.0 vs. O: 24.9 ± 3.9 units; $p < 0.05$) to a greater extent than the older men. Renal vascular resistance was higher in the older men throughout the protocol, but no differences between groups were observed in response to tilting. In addition, CVP (Y: -3.3 ± 0.8 vs. O: -2.2 ± 0.6 mmHg; $p < 0.05$) and SV (Y: -51 ± 5 vs. O: -39 ± 5 ml/bt; $p < 0.05$) decreased more in the young men, but HR increased more (Y: 18 ± 3 vs. O: 9 ± 4 beats/min; $p < 0.05$), so that the fall in Q_c was similar between the two groups upon assumption of the upright posture.

Quantitatively, heat stress during tilting did not significantly alter the differential responses between the two groups; however, only 4 of the young men and 6 of the older men were able to finish the second tilt without becoming pre-syncope. In summary, the older men relied on a greater increase in splanchnic vascular resistance to compensate for a reduced ability to constrict the skin and muscle circulations (as determined by changes in forearm vascular resistance) during head-up tilting.

From this investigation we suggest that the augmentation of cardiac filling via splanchnic vasoconstriction assumes a preponderant role over muscle and skin vasoconstriction during purely passive orthostatic conditions. The augmentation of splanchnic vasoconstriction via skin cooling will subsequently become an important focus in our future investigations.

Approximately 500,000 Americans exhibit some form of orthostatic intolerance. The prevalence of this disorder increases with aging, suggesting that this problem will increase with aging of the American population. Frank hypovolemia, as a consequence of space flight, hemorrhage, renal dialysis, or aging, exacerbates the problem as does a failure to redistribute blood from the splanchnic region, such as that occurring with younger people during passive heating, some older populations with post-prandial hypotension, and patients with autonomic dysfunction. We believe that by addressing this mechanism, our findings translate between elderly people, some patients, and space sojourners; moreover, skin cooling should prove to be an effective means to distribute blood volume centrally to improve orthostatic tolerance.

FY97 Publications, Presentations, and Other Accomplishments:

Levine, B.D., Zuckerman, J.H., and Pawelczyk, J.A. "Cardiac atrophy after bed-rest deconditioning: A non-neural mechanism for orthostatic intolerance." *Circulation*, 96, 517-525 (1997).

Pawelczyk, J.A. "Cardiovascular adaptations to spaceflight: A terrestrial perspective." Keynote address at the 5th Annual Research Appreciation Day, University of North Texas Health Science Center (March, 1997).

Pawelczyk, J.A. "Contemporary neuroscience during spaceflight: The Neurolab Space Shuttle Mission." Keynote address at the 15th annual Houston Conference on Biomedical Engineering Research (February, 1997).

Pawelczyk, J.A., Pawelczyk, R.A., Warberg, J., Mitchell, J.H., and Secher N.H. "Cardiovascular and catecholamine responses to static exercise in partially curarized humans." *Acta Physiol. Scand.*, 160, 23-28 (1997).

Shi, X., Pawelczyk, J.A., Zuckerman, J.H., Raven, P.B., and Levine, B.D. "Bed-rest (BR) enhances calf vasodilator response to saline infusion (SI) induced central hypovolemia." (Abstract) *Med. Sci. Sports Exercise*, 28, S173 (1996).

Shi, X., Pawelczyk, J.A., Zuckerman, J.H., Raven, P.B., and Levine, B.D. "Forearm vascular resistance (FVR) response to change in cardiac volume: Effect of physical deconditioning." (Abstract) *Physiologist*, 39, A18 (1996).

Zhang, R.H., Zuckerman, J.H., Pawelczyk, J.A., and Levine, B.D. "Effects of head-down tilt bedrest on cerebral hemodynamics during orthostatic stress." *J. Appl. Physiol.* (In Press).

Zhang, R., Zuckerman, J.H., Pawelczyk, J.A., and Levine, B.D. "Regulation of cerebral blood flow after simulated microgravity." (Abstract) *Med. Sci. Sports Exercise*, 28, S173 (1996).

Zuckerman, J.H., Zhang, R., Pawelczyk, J.A., and Levine, B.D. "Heart rate and blood pressure variability after bedrest deconditioning." (Abstract) *Med. Sci. Sports Exercise*, 28, S173 (1996).

Zuckerman, J.H., Zhang, R., Pawelczyk, J.A., and Levine, B.D. "Plasma volume effects on bed rest deconditioning related baroreflex impairment." (Abstract) *Circulation*, 94(8), I-490 (1996).

Pulmonary Deposition of Aerosols in Microgravity

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Initial Funding Date: 1995

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Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$234,936

Task Description:

The intrapulmonary deposition of airborne particles (aerosol) in the size range of 0.5 to 5 microns is primarily due to gravitational sedimentation. In the microgravity (μ G) environment, sedimentation is no longer active, and thus there should be marked changes in the amount and site of the deposition of these aerosols. We propose to study the total intrapulmonary deposition of aerosol spanning the range 0.5 to 5 μ in the KC-135 at both μ G and at 1.8-G. This will be followed by using boli of 1.0 μ aerosol, inhaled at different points in a breath to study aerosol dispersion and deposition as a function of inspired depth. The results of these studies will have application in better understanding of pulmonary diseases related to inhaled particles (pneumoconioses), in studying drugs delivered by inhalation, and in understanding the consequence of long-term exposure to respirable aerosols in long-duration space flight.

In FY 1997, we performed the bolus dispersion and deposition studies planned in our original proposal. The total deposition system developed for the previous studies (described in the FY1996 Task Summary) was modified to include pneumatically operated sliding valves that allowed for the breathing path of the subject to be switched under computer control at precisely defined points in a test breath. Subjects were asked to perform a standardized respiratory maneuver in which they exhaled to RV, inhaled at \sim 0.4 l/sec to a volume approximately 1 liter above the FRC in 1G, and then exhaled at \sim 0.4 l/sec to RV. This maneuver was performed in both the microgravity and hypergravity portions of the KC-135 flight profile. During the controlled inspiration, the pneumatically operated valves were triggered to deliver a 70ml bolus of particle laden air at a given point in the inspiration. The point was set at a number of predefined penetration volumes. For example, if the valves were triggered very late in the inspiration, then only a small volume of particle free air would follow the bolus and the resulting lung penetration volume was very small, with particles staying mostly in the central airways. In contrast, if the valves were triggered early in the inspiration, the resulting lung penetration volume of the bolus was large and the particles were delivered to the small airways and alveolar regions of the lung. Several penetration volumes between 150 ml and 1500 ml were chosen and studied.

We had originally planned to perform bolus studies using 1 micron particles. In March 1997, we flew the bolus system for the first time and achieved excellent results. During this set of flights, it became apparent that we

were in fact able to perform our tests at closer intervals than we had originally anticipated, and as a consequence during the subsequent flights in July and August 1997, we succeeded in completely mapping the regional intrapulmonary deposition of 0.5, 1, and 2 micron particles in normal gravity, microgravity, and hypergravity. This is a significant increase in the amount of scientific data we were able to collect beyond that originally proposed.

In FY 1997 we completed the manuscript reporting the total deposition studies performed in FY 1996 and submitted this to the *Journal of Applied Physiology*. The paper received a favorable review and has been accepted for publication. The enhanced deposition reported in that paper, which we attributed to enhanced diffusion, has sparked a considerable degree of interest, especially from the group of Dr. James P. Butler at the Harvard School of Public Health. Butler and Tsuda have recently published a report of a novel new mechanism of convective mixing in the lung periphery they refer to as "Stretching and Folding." We intend to pursue the idea that stretching and folding is responsible for our unexpected results. The microgravity environment of the KC-135 provides us with a unique opportunity to do this as in the absence of gravity we can examine the behavior of the aerosol particles without the confounding effects of gravitational sedimentation.

At this point we have completed the experimental phase of the proposed research with approximately 200% of the data we intended to collect. We will spend much of the next fiscal year analyzing and interpreting the results. We intend to pursue some modeling studies to shed light on the results.

This program seeks to obtain a better understanding of the processes of deposition of inhaled particles in the human lung. Inhaled particles deposit on the walls of the airways and gas exchange regions of the lung by three mechanisms: impaction of large particles, sedimentation of medium sized particles, and movement by diffusion of the smallest particles. Particle deposition is important in many diseases that result from working in dusty environments, e.g., silicosis and asbestosis among many. Further, the deposition of particles in the lung is very important in the delivery of many therapeutic agents (e.g., the metered dose inhalers used by asthmatics). In these cases, the site and efficiency of deposition of the medium sized particles is critically important for the efficacy of the drug therapy. Since sedimentation is a gravitational process, by studying the changes in deposition of test particles in the absence of gravity, we hope to gain a better understanding of the entire process of deposition. This can then be fed back to provide better aerosol generation, targeting more specific sites in the lung. The process of deposition in the weightless environment is also clearly important for the people that will be continuously exposed to suspended particles in the Space Station environment.

Our data to date suggest that alveolar deposition of small particles may in fact be much higher than originally anticipated. Since the alveoli are highly susceptible to damage by inhaled substances, this may have a fundamental bearing on the development of some environmentally based pulmonary diseases. For example, it is now believed that much of the rise in asthma prevalence may be due to the inhalation of small (< 2.5 micron) particles, and new federal standards are being proposed to control the levels of these particles. The recent findings in our studies of total deposition emphasize the need for direct measurements of regional deposition and dispersion, studies using inhaled boluses of particles. We have already completed these measurements as part of this program.

FY97 Publications, Presentations, and Other Accomplishments:

Darquenne, C., Paiva, M., West, J.B., and Prisk, G.K. "Deposition of aerosols in the human lung at different gravity levels." *Am. J. Resp. and Crit. Care Med.*, 155, A955 (1997).

Darquenne, C., Paiva, M., West, J.B., and Prisk, G.K. "Effect of microgravity and hypergravity on deposition of 0.5 to 3 mm-diameter aerosol in the human lung." *J. Appl. Physiol.* (In Press).

Darquenne, C., Paiva, M., West, J.B., and Prisk, G.K. "Intrapulmonary aerosol deposition in micro- and hypergravity: Evidence for enhanced alveolar deposition." *J. Aerosol Med.*, 10, 252 (1997).

Mechanisms of Microgravity Effect on Vascular Function

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Funding:

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Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$192,021

Task Description:

The proposed study addresses the effects of microgravity on vascular function with particular relevance to the problem of orthostatic intolerance experienced by astronauts on re-entry following space flight. It is clear that the decreases in plasma volume and baroreceptor reflex responsiveness during space flight contribute to, but do not fully account for, re-entry orthostatic intolerance. The proposed study will investigate the largely unexplored possibility that adaptive changes in vascular smooth muscle and/or associated sympathetic or other innervating nerve terminals occur during space flight (zero gravity) that result in decreased responsiveness of the vasculature. Microgravity is simulated using the hindlimb unweighted (HU) rat, and the following vessels are removed from HU and paired control rats for *in vitro* analysis: abdominal aorta, carotid and femoral arteries, and jugular and femoral veins. Three mm-long rings of vessel are mounted in tissue baths for the measurement of either isometric contraction or relaxation of precontracted vessels. The isolated mesenteric vascular bed is perfused for the measurement of changes in perfusion pressure as an index of arteriolar constriction or dilation. The justification for this work is that it will explore a potential major mechanism underlying orthostatic intolerance, thereby providing a basis for the development of more effective countermeasures.

Previous progress reports have shown that arteries from hindlimb unweighted (HU; simulated microgravity) rats exhibit markedly decreased maximal contractions to norepinephrine (NE). The following are the findings during the present review period.

Time course of HU effect. The normal duration of HU treatment used is 20 days. However, the maximal contraction to NE was reduced after 3 days of HU treatment in aorta and after only 1 day in carotid artery. In contrast, 20 days of HU treatment were required to reduce contractility in the femoral artery.

Effect of HU on NE, 68 mM K⁺ and serotonin-induced contractions. HU treatment reduced the maximal contractions to NE and 68 mM K⁺ in aorta, carotid, and femoral arteries. However, HU treatment had no effect on the maximum contraction of these vessels to serotonin.

HU effect on endothelium in carotid artery. Endothelium removal restored the contractility of carotid arteries from 20 day HU treated rats to that of control carotid arteries. Exposure of endothelium-intact HU

arteries to L-nitroarginine methylester (L-NAME) to block endothelial constitutive nitric oxide synthase (ecNOS) had the same effect. Precontracted carotid arteries from HU rats were 5-fold more sensitive to the relaxing effects than those from control rats. Finally, Western blot analysis revealed that carotid arteries from 20 day HU rats possessed significantly more ecNOS protein mass than control carotid arteries. Taken together, these results suggest that HU treatment results in the upregulation of ecNOS in the carotid artery, resulting in higher levels of basal and, possibly, stimulation-induced nitric oxide formation. In turn, this accounts for a reduced capacity of the carotid artery to contract to norepinephrine.

HU effect on nitric oxide-related processes in femoral artery. Endothelium removal had no effect on the femoral artery. HU treatment still reduced the maximal contraction to NE. When Western blot analysis of ecNOS was conducted, inducible NOS (iNOS) was also assessed. Surprisingly, HU treatment was found to increase iNOS in femoral, but not carotid artery. Two functional tests were performed to assess the possible role of iNOS in the reduced contractile capacity of femoral arteries from HU rats. Femoral arteries were exposed to 0.3 mM arginine, the substrate for iNOS formation of nitric oxide. These vessels were then contracted with NE in the presence and absence of the selective iNOS inhibitor, aminoguanidine. The HU vessels in the absence of aminoguanidine exhibited a reduced contractile response to NE while those in the presence of aminoguanidine contracted to the same level as control vessels. In a second test, precontracted femoral artery rings were exposed to 3 mM arginine. Arginine caused a significant relaxation in HU-derived vessels but had no effect in control vessels. In addition, aminoguanidine blocked the arginine-induced relaxation in the HU-derived vessels. Taken together, these results demonstrate that HU treatment induces iNOS in femoral artery, and that, in the presence of L-arginine, the increase in iNOS can account for the hyporesponsiveness of femoral artery to NE.

HU effect on blood pressure responses *in vivo*. In preliminary studies, HU and control rats received intravenous injections of NE and aminoguanidine. The blood pressure response in the HU rats was less than half that in the control rats. In contrast, the elevation of blood pressure by the iNOS inhibitor, aminoguanidine, was three times greater in HU compared to control rats. This strongly suggests that, *in vivo*, elevation of vascular iNOS contributes importantly to the hyporesponsiveness to NE in HU rats.

Future experiments. The roles of ecNOS and iNOS in simulated microgravity will be addressed in the competing renewal application for this project. In the remaining time covered by the present funding, experiments will be carried out to achieve the following: 1) characterize the effects of HU treatment on the cerebrovasculature, using the middle cerebral artery as a model; 2) determine the effect HU treatment on the cellular mobilization of calcium from the external medium and from intracellular stores, with simultaneous measurement of contraction using vascular rings; and 3) determine the second messenger biochemical steps mediating the responses to NE and serotonin.

This research seeks to understand the malady of postural intolerance experienced by space-adapted astronauts on return to the Earth's gravitational field. This research will yield new information concerning biological mechanisms in the vascular system by which exposure to microgravity depresses vascular contractility, contributing to postural intolerance. It is the long-term goal of this research to use the new understanding of these biological mechanisms to develop specific therapies to prevent microgravity-induced postural hypotension and intolerance.

Concerning humans on Earth, there are several maladies in which the patient experiences postural intolerance or hypotension. These include dysautonomia, diabetes, and any malady that requires long-term, continuous bedrest. Some of the same mechanisms that underlie microgravity-induced postural intolerance may operate in these conditions as well. The specific therapies developed to help astronauts may also be found to be beneficial in these patients.

FY97 Publications, Presentations, and Other Accomplishments:

Purdy, R.E., Sara, D., and Loyola, S.M. "Time course of simulated microgravity effect on vascular contractility." *FASEB J.*, 11(3), 1402 (1997).

Purdy, R.E., Sara, D., Duckles, S.P., and Krause, D.N. "Simulated microgravity impairs vascular contractility." *Proc. West. Pharmacol. Soc.* 40:25-27 (1997).

Sara, D., Duckles, S.P., Krause, D.N., Loyola, S.M., and Purdy R.E. "Simulated microgravity reduces vascular contractility: Role of vascular smooth muscle versus endothelium." (Abstract) *FASEB J.*, 11(3), 1401 (1997).

Carotid Baroreflex Function During Prolonged Exercise

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Task Description:

Astronauts are often required to work (exercise) at moderate to high intensities for extended periods while performing extra-vehicular activities (EVA). Although the physiologic responses associated with prolonged exercise have been documented, the mechanisms involved in blood pressure regulation under these conditions have not yet been fully elucidated. An understanding of this issue is pertinent to the ability of humans to perform work in microgravity and complies with the emphasis of NASA's Space Physiology and Countermeasures Program.

Prolonged exercise at a constant workload is known to result in a progressive decrease in mean arterial pressure (MAP) concomitant with a decrease in stroke volume and a compensatory increase in heart rate. The continuous decrease in MAP during the exercise raises the question as to whether there is a loss of baroreflex regulation of arterial blood pressure. We propose that with prolongation of the exercise to 60 minutes, progressive increases in central command reflect a progressive upward resetting of the carotid baroreflex (CBR) such that the operating point of the CBR is shifted to a pressure below the threshold of the reflex rendering it ineffectual in correcting the downward drift in MAP. In order to test this hypothesis, experiments have been designed to uncouple the global hemodynamic response to prolonged exercise from the central command mediated response via continuous maintenance of cardiac filling volume by intravenous infusion of a dextran solution. As the type of work (exercise) performed by astronauts is inherently arm and upper body dependent, we will also examine the physiologic responses to arm and leg cycling to determine the effects of increases in active muscle mass during prolonged arm ergometry exercise in the supine positions with and without low level (-10 mmHg) lower body negative pressure to mimic spaceflight related decreases in cardiac filling volumes.

During FY97, we completed two investigations which have been submitted for publication.

Investigation 1: Examined the resetting of the carotid arterial baroreflex during dynamic arm and leg exercise at 50%, 75%, and 100% of maximal oxygen uptake. The data clearly demonstrated that as the intensity of exercise increased by the addition of arm to leg exercise, the arterial baroreflex was reset to function at the prevailing exercise blood pressure. Furthermore, the operating pressure of the reflex moved toward the threshold of the reflex with increasing exercise intensity. By adding arm exercise to leg exercise at the same intensity of exercise

as leg exercise alone, the baroreflex was reset vertically upward indicating a greater effect of the exercise pressor reflex.

Investigation 2: This investigation was designed to uncouple the hemodynamic physiologic effects of thermoregulation from the effects of a progressively increasing central command activation during prolonged exercise. Subjects performed two one-hour bouts of leg cycling exercise with (a) no intervention and (b) continuous infusion of a dextran solution to maintain central venous pressure constant at the 10 minute pressure. Volume infusion resulted in a significant reduction in the decrement in mean arterial pressure (MAP) seen in the control exercise bout, 6.7 ± 1.8 versus 11.6 ± 1.3 mmHg, respectively. However, indices of central command such as heart rate and oxygen uptake rose to a similar extent during both exercise conditions. In addition, the carotid baroreflex (CBR) stimulus-response relationship as measured using the neck pressure neck suction technique was reset from rest to 10 minutes of exercise and was further reset from 10 to 50 minutes of exercise in both exercise conditions with the operating point being shifted toward the reflex threshold. We concluded that the progressive resetting of the carotid baroreflex and the operating point renders the reflex ineffectual in counteracting the continued decrement in MAP that occurs during the prolonged exercise.

These findings have answered two of the questions raised by the revised work task. However, other questions remain to be answered. These include: i) What is the effect of arm exercise alone on cardiovascular drift and baroreflex resetting? and ii) Does prolonged exercise alter the regulation of vasomotion?

The initial investigations affect the proposed future work positively in that we have developed the foundation upon which we can investigate the arm exercise protocols.

The current work tasks were designed to address some fundamental regulatory mechanisms which have been recognized to pertain to moderate work load performed for one hour or more both on Earth and in space. It is our proposal that if we can understand the mechanism associated with cardiovascular drift, we should be able to devise a benign pharmacological or mechanical means to reduce the cardiovascular stress on the individual.

FY97 Publications, Presentations, and Other Accomplishments:

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The Sympathetic Nervous System in the Anemia of Weightlessness

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Funding:

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Solicitation: not available

Initial Funding Date: 1994

Expiration: 1997

Students Funded Under Research: 3

Post-Doctoral Associates: 2

FY 1997 Funding: \$0

Joint Agency Participation: NIH

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Mild anemia has been noted during both American and Soviet space flights. A fall in red blood cell mass of approximately 15% has been seen within weeks. Although some stabilization may occur after two months, significant anemia seems to persist. New information on this process has come from results of the SLS-1 mission.

A number of investigations of potential causes have been carried out. However, the role of the sympathetic nervous system as a contributing cause for this reduction in red cell mass has not yet been addressed. Erythropoietin production is partly governed by sympathetic stimulation via actions of epinephrine and norepinephrine on β -adrenoreceptors.

In preliminary studies, we have discovered that patients with low levels of circulating norepinephrine have suppressed erythropoietin production and a corresponding anemia, which may be mild to moderate in severity. In healthy subjects, circulating norepinephrine is low with supine posture and 2-3 fold higher during upright posture. A diurnal pattern of blood erythropoietin has been recently described. Although the cause of this pattern (high erythropoietin during the day, low at night) was not recognized, the pattern is congruent with prevailing norepinephrine levels. We propose that relatively low circulating norepinephrine levels in microgravity (and also in patients largely confined to bed because of chronic illness) lead to inadequate levels of circulating erythropoietin, which in turn contribute to the observed anemia.

Studies to test this hypothesis will include manipulations of norepinephrine and erythropoietin by physiological and pharmacological interventions, monitoring of the relevant variables during bedrest, and systematic studies to assess the potential of simple countermeasures such as sympathomimetic amine preparations to correct the erythropoietin deficiency and anemia. These studies have potential implications for patients chronically at bedrest which may be similar to those for astronauts and, if our hypotheses are correct, may lead to changes in the management of anemia produced by chronic bedrest.

Studies of norepinephrine manipulation and anemia are continuing. A norepinephrine-like agent (ephedrine) currently marketed is also being used to study these effects. In preliminary work, we studied the autonomic pharmacology of ephedrine. Ephedrine has been used in an effort to achieve weight control for many years. It is widely available in prescription and non-prescription drugs as well as in herbal products such as *ma huang*. There are limited data on its true efficacy in altering energy balance in human subjects under carefully controlled conditions.

We assessed the acute effect of ephedrine on 24-hour energy expenditure in 10 healthy volunteers (6 males, 4 females, 30.9 ± 4.9 yrs (S.D.), 74.5 ± 12.4 kg, 175.2 ± 9.1 cm, BMI 24.1 ± 2.49 kg/m²) in a double blind, randomized, placebo-controlled, 2-period crossover study. Each subject was given ephedrine (50 mg) or placebo t.i.d. during each of two 24-hour periods (ephedrine and placebo) in a whole-room indirect calorimeter which accurately measures minute-by-minute energy expenditure and physical activities reflected in mechanical work. In addition, each subject received placebo t.i.d. during a baseline 24-hour period in the calorimeter.

There were no significant differences in 24-hour or sleeping energy expenditure or in mechanical work between baseline and placebo periods. Twenty-four hour energy expenditure was 3.6% greater (2143 ± 311 vs 2067 ± 322 kcal, $p < 0.05$) with ephedrine than with placebo. Twenty-four hour mechanical work was not significantly different between the ephedrine and placebo periods. Most of the elevated energy expenditure with ephedrine occurred during sleep, where the energy expenditure was 8.4% greater with ephedrine than with placebo ($p < 0.005$). Mechanical work was quite small during sleep (0.001 kcal/min), but did increase during sleep after ephedrine treatment (0.002 kcal/min) ($p < 0.05$). With adjustment for mechanical work, basal energy expenditure increased during sleep by 7.3% ($p < 0.01$).

There is an acute effect of ephedrine (50 mg t.i.d.) on energy balance in normal human subjects but the effect is modest. More information concerning the safety profile and the chronic effect of ephedrine on energy balance would be required to justify its role in weight reduction programs.

This research was undertaken in an effort to better understand how an absence of gravity might lead to anemia. This original question is considered relevant to patients at chronic bedrest. These studies are continuing, and we should understand the relevance of this to the anemia of chronic disease by the end of the study. The unexpected discovery that in patients with orthostatic intolerance (mitral valve prolapse, chronic fatigue syndrome, and other disorders which fall into this framework), there is a very significant increase in loss of fluid from the vasculature during upright posture. This observation, made possible by the NASA support of the anemia studies, may have important implications for the future management of patients with orthostatic intolerance. It is believed that approximately 500,000 Americans suffer from orthostatic intolerance. No mechanism for this has ever been clearly identified. The documentation in our study of a dynamic orthostatic hypovolemia in these subjects was unanticipated but will probably alter how we understand and treat these patients in the future.

FY97 Publications, Presentations, and Other Accomplishments:

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Jordan, J., Shannon, J., and Robertson, D. "The physiological conundrum of hyperadrenergic orthostatic intolerance." *Chinese J. Physiol.*, 40, 1-8 (1997).

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Mosqueda-Garcia, R., Furlan, R., Fernandez-Violante, R., Desai, T., Snell, M., Jarai, Z., Ananthram, V., Robertson, R.M., and Robertson, D. "Sympathetic and baroreceptor reflex function in neurally-mediated syncope evoked by tilt." *J. Clin. Invest.*, 99, 2736-2744 (1997).

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Shannon, J. and Robertson, D. "Orthostatic intolerance" in "Current Therapy of Endocrinology and Metabolism." Edited by: Bardin, C.W. Philadelphia: Mosby-Year Book, pp 197-203 (1996).

Shannon, J., Jordan, J., Costa, F., Robertson, R.M., and Biaggioni, I. "The hypertension of autonomic failure and its treatment." *Hypertension* (In Press).

Wrenn, C.C., Picklo, M.J., Lappi, D.A., Robertson, D., and Wiley, R.G. "Central noradrenergic lesioning using anti-DBH-saporin: Anatomic findings." *Brain Res.*, 740, 175-184 (1996).

Mechanisms of Antiarrhythmic Drug Action

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Students Funded Under Research: 0

Post-Doctoral Associates: 2

FY 1997 Funding: not available

Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Task Description:

The overall goal of this project is to determine the mechanisms underlying the highly variable effect of antiarrhythmic drug therapy. In some patients, treatment with antiarrhythmic drugs can be lifesaving, whereas in others the same drugs may be ineffective or even provoke life-threatening arrhythmias. Clinical and *in vitro* evidence strongly suggests that one factor which modulates response to antiarrhythmic drug therapy is autonomic tone, and projects investigating the effects of activation of intracellular signalling mechanisms on ion channel function at the molecular and cellular levels are in place in this program.

This proposal has two major goals: first, to develop models of sympathetic inhibition relevant to the study of microgravity and, second, to evaluate the effects of such sympathetic inhibition on response to antiarrhythmic drug therapy. Despite the obvious stresses accompanying space travel, plasma norepinephrine is remarkably decreased in astronauts. Thus, the first aim of this proposal will be to develop models of the reduction of sympathetic activity produced by simulated microgravity. Three approaches, the norepinephrine release inhibitor guanadrel, the central α_2 agonist clonidine, and prolonged bedrest, will be assessed. State-of-the-science techniques to assess sympathetic function, including measurements of norepinephrine spillover and clearance, spectral analysis of heart rate, and direct measurement of sympathetic nerve traffic with microneurography, will be used. Preliminary studies strongly suggest that basal QT interval and QT prolongation induced by drugs such as quinidine are influenced by sympathetic activity. Therefore, the second aim of this proposal is to use the clinical models of reduced sympathetic activity to test the hypothesis that sympathetic inhibition will exaggerate the QT prolonging effects of quinidine in human subjects.

This research is highly complementary to the aims of the extant Program Project. By developing new techniques to study sympathetic inhibition, the research will not only expand our understanding of the physiologic adjustments to space travel, but also provide new tools to study the effect of modulation of autonomic tone on cardiac electrophysiology and its response to antiarrhythmic drug action.

During the final year of funding (which includes FY97), we made significant contributions to the understanding of orthostatic intolerance, a condition that affects many of the astronauts upon returning to Earth. Orthostatic intolerance (OI) is a frequent and debilitating autonomic condition in young adults. Its neurohumoral and hemodynamic profiles suggest possible alterations of postural sympathetic function and of baroreflex control of heart rate (HR).

In 16 OI patients and 16 healthy volunteers, intra-arterial blood pressure (BP), EKG, central venous pressure (CVP) and muscle sympathetic nerve activity (MSNA) were recorded at rest and during 75° tilt. Spectrum analysis of RR interval and systolic arterial pressure (SAP) variabilities provided indices of sympatho-vagal modulation of SA node (LF/HF) and of sympathetic vasomotor control (LF_{SAP}). Baroreflex mechanisms were assessed (1) by the slope of the regression line obtained from changes of RR interval evoked by pharmacologically induced alterations in BP, and (2) by the index, obtained from cross-spectral analysis of RR and SAP variabilities. At rest, HR, MSNA, LF/HF and LF_{SAP} were higher in OI while BP and CVP were similar in both groups. During tilt, BP did not change and CVP fell by the same extent in the two groups; the increase of HR and LF/HF was more pronounced in OI. Conversely, the increase of MSNA was lower in OI than in controls at rest; tilt reduced similarly in both groups.

OI is characterized by an overall enhancement of noradrenergic tone at rest and by a blunted postganglionic sympathetic response to standing with a compensatory cardiac sympathetic overactivity. Baroreflex mechanisms maintain their functional responsiveness. These observations suggest that in OI, the functional distribution of central sympathetic tone to the heart and vasculature is abnormal.

A complex but crucial relationship exists between blood volume and blood pressure in human subjects. Heretofore, it has been recognized that in essential hypertension, renovascular hypertension, and pheochromocytoma, the relationship between plasma volume and diastolic blood pressure is an inverse one. This phenomenon has not been studied in individuals with OI.

We tested the hypothesis that the relationship previously found between plasma volume and diastolic blood pressure in pressor states would also hold in orthostatic intolerance. We studied 16 patients with a history of symptomatic orthostatic intolerance associated with an elevation in plasma norepinephrine in the upright posture and hypovolemia in 9 patients and normovolemia in 7 patients.

Our studies demonstrate an inverse relationship between plasma volume and diastolic blood pressure in patients with orthostatic intolerance. This finding also holds for the change in diastolic blood pressure in response to upright posture. In this relationship, patients with orthostatic intolerance with high plasma norepinephrine resemble those with essential hypertension, renovascular hypertension and pheochromocytoma. We conclude that in a variety of conditions at both ends of the blood pressure spectrum, the seemingly paradoxical association of hypovolemia and diastolic blood pressure is preserved.

In related studies, we assessed local norepinephrine (NE) spillover (NSO) in arms and legs of 10 patients with OI (criteria: orthostatic tachycardia > 30 beats/min/ frequent presyncopal symptoms; and upright plasma NE > 600 pg/ml) and compared them to 8 matched controls. Invasive blood pressure (brachial artery and femoral artery) was used to determine local NE; venous occlusion cuff plethysmography was used to determine forearm and leg blood flow. Venous cannulae (brachial vein and femoral vein) were inserted to measure venous NE. The traditional tracer dilutions technique was used to determine the local NSO. We established the local NSO of the arm and the leg in each individual before and after a decrease in systolic blood pressure of 20 mmHg with systemic infusion of nitroprusside (NTP), an increase in the systolic blood pressure of 20 mmHg with systemic infusion of tyramine (TYR, NE-releasing effect) and a painful stimulus with the cold pressor test (CPT). The baseline NSOs were significantly lower in the arms and in the legs of patients compared to controls [Arms: patients vs controls: 2.5 ± 0.2 vs 5.5 ± 0.6 , 2.7 ± 0.4 vs 3.9 ± 0.4 , 2.6 ± 0.3 vs 4.4 ± 0.7 ng/min before TYR, NTP and the CPT, respectively ($p < 0.001$ before TYR, $p < 0.03$ before NTP and $p < 0.005$ before CPT)]; [Legs: patients vs controls: 1.4 ± 0.2 vs 2.7 ± 0.4 , 1.5 ± 0.2 vs 3.2 ± 0.4 , 1.1 ± 0.15 vs 2.5 ± 0.55 ng/min before TYR, NPT and CPT, respectively ($p < 0.02$ before TYR, NPT, and CPT, respectively ($p < 0.02$ before

TYR, $p < 0.001$ before NTP and $p < 0.025$ before CPT)]. Also, the increment in the local NSO of the arms tended to be lower but did not reach statistical significance, while NSO of the legs of patients was maintained significantly lower after each stimulus compared to controls [Legs: patients vs controls: 0.1 ± 0.2 vs 2.0 ± 0.7 , 0.3 ± 0.25 vs 2.1 ± 0.4 , 0.03 ± 0.3 vs 1.3 ± 0.4 mg/min before TYR, NTP and the CPT, respectively ($p < 0.03$ after TYR, $p < 0.01$ after NTP and $p < 0.025$ after CPT)]. In conclusion, these results provide strong evidence that partial dysautonomia underlies idiopathic orthostatic intolerance in the population studied.

This research will provide better understanding of the pathophysiologic changes produced by microgravity and may also improve our understanding of disease states such as autonomic dysfunction and orthostatic intolerance. We are exploring the notion that changes in autonomic function affect the action of antiarrhythmic drugs which should allow us to better define mechanisms of reflex cardiovascular function. Changes in sympathetic function often are required for adaptation of living organisms to a new environment. Developing Earth-based models for changes produced by space travel will allow us to be better prepared to design countermeasures.

FY97 Publications, Presentations, and Other Accomplishments:

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Optimization of a Biomechanical Countermeasure for Disuse Osteopenia

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Initial Funding Date: 1997

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

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Task Description:

Microgravity-induced bone loss represents a formidable hurdle to man's extended presence in space. Considering the biomechanical etiology of this osteopenia, a "surrogate" for the absence of gravity seems an expedient countermeasure to combat this loss. Preliminary work in this laboratory has demonstrated that short periods (< 10 minutes) of extremely low magnitude (< 20 microstrain) mechanical loading can be osteogenic if applied at a high frequency (15 to 60 Hz). As these high frequency, low magnitude strains comprise a principal constituent of a bone's functional strain history, these mechanical events may well represent a dominant determinant of bone mass and morphology. We hypothesize that small increases in this low magnitude, high frequency strain domain, introduced non-invasively into the skeleton via vibration, will stimulate an increase in bone mass without compromising bone quality. Considering these strain levels are < 1/500th of those which may cause damage to the tissue, we believe these signals also hold great potential as a countermeasure for microgravity-induced bone loss.

In Phase 1 of the protocol, 300g male rats will be placed in a randomized, partial 5x4x3 factorial experimental design to evaluate the interdependent efficacy of frequency (10, 20, 40, 80, or 160 Hz), duration (5, 10, 20, or 40 min.), and intensity (0.2, 0.4, or 0.8g-force) to augment the cortical and trabecular bone of the tibia following 28 days of daily mechanical stimulation. In Phase 2, using the rat tail-suspension model of disuse, differential mRNA display will be employed to study alterations in gene expression. This method utilizes the polymerase chain reaction (PCR) and will facilitate the comparison, isolation, and identification of specific genes stimulated or down-regulated in the bone tissue by the diminished functional activity. In Phase 3, the most osteogenic mechanical signals identified in Phase 1 will be used to determine if changes in mRNA expression caused by tail-suspension can be normalized by short durations (< 10 minutes) of mechanical stimulation. These experiments may yield new insights into the molecular mechanisms by which mechanical factors control bone morphology, as well as lead to a novel treatment for bone loss due to long-term space flight, and the more mundane, but more prevalent, Earth-bound types I&II osteopenia.

This work began in March, 1997. This brief report of progress reflects work six months into the protocol. We have begun to evaluate the cortical and trabecular bone response to 30 days exposure to low magnitude, high frequency mechanical stimuli. Twenty groups (n=6 in each group) in the clustered factorial, evaluating intensity, frequency and duration, have completed the loading regimen. The majority of the bone samples are now in processing, while the bone response from a few groups have been analyzed. Thus far, we have completed the analysis of: (1) zero time control animals; (2) long term control animals; (3) 0.5g @90Hz for 80 minutes; (4) 0.5g @ 45Hz for 40 minutes; and (5) 0.5g @ 22.5Hz for 10 minutes.

While the data are preliminary, we are seeing significantly significant increases in bone parameters of the proximal tibial metaphysis of each of the loading groups. This includes parameters such as labeled surface, mineral apposition rate, and bone formation rate. We have successfully extracted RNA from the contralateral tibiae of these animals, and are currently using differential display PCR to evaluate the mechanically sensitive genes which may participate in this osteogenic response. Finally, we have just begun the tail suspension studies, with the goal of identifying the genes involved in inhibiting the formation response.

Identifying a biomechanical prophylaxis for the inhibition and/or reversal of osteopenia would be a great benefit to the American populus. Considering there are over 20 million people suffering from this disease, and no pharmaceutical prophylaxis has proven universally effective or without side effect, the potential impact of an efficacious intervention therapy that is based on a non-invasive stimulation of the musculo-skeletal system is enormous. This work will serve to identify the critical parameters of the mechanical regimen, and evaluate the efficacy of a relatively simple, safe prophylaxis for bone disease. Further, the efforts to isolate and identify the genes involved in this process may open up unique avenues for drug development and discovery.

Architecture and Mechanical Function in Bone with Recovery from Disuse Osteoporosis

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Students Funded Under Research: 1
FY 1997 Funding: \$194,520

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Expiration: 1999
Post-Doctoral Associates: 1

Task Description:

Disuse results in a loss of bone mass estimated to be an order of magnitude greater than that in any other metabolic disorder of bone. Reversal of an extant osteoporosis is thought to result in recovery of bone mass but not the restoration of microarchitecture or the replacement of lost trabecular elements. Specifically, it is thought that restoration of bone mass after osteoporosis occurs through compensatory thickening of remaining trabecular elements; restoration of trabecular bone microarchitecture (lost trabeculae, interconnectedness of trabecular elements) is thought not to occur. However, there is very little direct information available to support or refute these assertions. The functional-mechanical consequences of having fewer thick trabeculae versus smaller, more numerous, interconnected trabecular elements have been the subject of extensive discussion, but again there is very little direct data on these structure-function relationships. Understanding the recovery potentials of the osteoporotic skeleton architecturally, mechanically, and biologically, has considerable clinical and functional significance.

Our recent studies show that during disuse-induced bone loss in the canine skeleton, there is a discrete, temporal separation of thinning of trabeculae from the later perforation and complete loss of trabecular elements. In the proposed experiments, we will take advantage of this sequence of bone changes in the development of disuse-induced bone loss to establish different architectural baseline points from which recovery of structure and mechanical function with reloading can be examined. We will use a cast immobilization of the canine forelimb to establish baseline points for remobilization. After remobilization, bone microarchitecture will be nondestructively evaluated using microcomputed tomography, and then tested mechanically to determine the mechanical integrity of bone after recovery from disuse.

During FY 97, animal subjects were initiated in long-term immobilization-remobilization portion of the experiments. Tissues from these experiments will be harvested and entered into the analytical phase of the project, beginning in FY98.

In addition, in FY97, microcomputed tomographic studies of three-dimensional bone architecture and histomorphometric analyses were completed for the initial cohort of immobilization bone specimens. The results

show that disuse osteoporosis develops through a uniform symmetric enlargement of existing pores. This is equivalent to the observation that the sequence of trabecular element deletions during age-related bone loss in humans is uniformly random. The anisotropy ratio (longest/shortest MIL) was unchanged by disuse in the current study, and the orientation of the principal axes was not changed. Accordingly, despite the loss of trabecular elements with long-term disuse, the overall plan of orientation remains unchanged, consistent with a uniformly random model for trabecular element deletion. These data have been written-up and will be presented at the 1998 meeting of the Orthopaedic Research Society.

Osteoporosis is a loss of bone which accompanies a number of skeletal processes, most notably the onset of menopause, decreased mechanical usage, and aging. In these instances, bone losses are characterized as deficits of bone volume or increases in tissue void space (e.g., thinning of bone cortices, trabecular rarefaction); they are considered osteoporoses. Qualitative changes in the bone matrix, such as changes in the relative mineral or organic fractions, are not typically implicated in the pathophysiology of osteoporosis.

Bone loss secondary to decreased mechanical usage follows space-flight, long-term immobilization, and prolonged bed rest, and may also be implicated in the local bone loss processes which accompany implant loosening. Significantly, disuse osteoporosis results in a loss of bone from all skeletal envelopes that is estimated to be an order of magnitude greater than that in any other metabolic disorder which affects bone. It is widely held that the osteoporosis resulting from decreased mechanical usage is reversible - with restoration of normal mechanical usage bone architecture and mass. However, there is substantial evidence which suggests that reversibility may not be possible or is at least markedly limited. The biological and architectural bases which allow complete versus limited reversal of disuse osteoporosis may have significant implications to the treatment and reversibility of other osteoporoses.

Hypodynamic states, such as space flight and immobilization, result in a loss of bone mass as well as dramatic changes in bone architecture. Recent studies in our laboratories and by others show that in trabecular bone these changes are caused by a combination of both thinning of trabeculae and by perforation of trabecular plates, resulting in complete loss of trabecular elements. Reversal of an osteoporosis is thought to result in recovery of bone mass but not the restoration of microarchitecture or the replacement of lost trabecular elements. Specifically, it is thought that restoration of bone mass after osteoporosis occurs through compensatory thickening of remaining trabecular elements. Restoration of trabecular bone microarchitecture (lost trabeculae, interconnectedness of trabecular elements) is thought not to occur. Surprisingly, however, there is very little direct information available to support or refute these assertions.

The functional-mechanical consequences of having fewer thick trabeculae versus smaller, more numerous, interconnected trabecular elements have been the subject of extensive discussion, but again there is very little direct data on these structure-function relationships. Understanding the recovery potentials of the osteoporotic skeleton, both biologically and architecturally, has considerable clinical and functional significance.

FY97 Publications, Presentations, and Other Accomplishments:

Schaffler M.B., Rabinovich, A., and Fyhire, D.P. "Programmatic loss of cancellous bone in disuse osteoporosis." *Trans Orthop. Res. Soc.* (In Press).

Vestibular Contributions to Post-Spaceflight Orthostatic Intolerance: A Parabolic Flight Model

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Funding:

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Responsible NASA Center: Johnson Space Center

Task Description:

Astronauts experiencing greater degrees of motion sickness during and/or after space flight often have poorer postflight orthostatic tolerance than their nonmotion sick (or less motion sick) crewmates, even when fluid losses attributable to nausea or emesis are minimal or reversed in the motion sick individuals. This observation is intriguing because: (1) data from multiple invasive animal studies clearly demonstrate that neuroanatomic connections and neurophysiologic relationships exist between vestibular and cardiovascular control areas in the central autonomic nervous system; and (2) data from noninvasive human studies also suggest that significant cardiovascular changes can occur during vestibular stimulation, particularly when motion sickness is induced.

In this study, seated parabolic flight on NASA's KC-135 aircraft was used as a stimulus to generate quantifiable motion sickness in susceptible test subjects. Changes in responses to various provocative autonomic cardiovascular stimuli (including Valsalva maneuvers, carotid-cardiac baroreflex studies, heart rate variability tests, and head-up tilt) after the onset of motion sickness were compared to the changes that occurred, if any, in nonmotion sick-susceptible subjects who experienced the same parabolic flight pattern.

The two principal objectives of this study were: (1) to investigate the relationship (correlation) between gravitationally-induced motion sickness and deficits in orthostatic tolerance, if any; and (2) to investigate the relationship between gravitationally-induced motion sickness and changes in autonomic cardiovascular function as determined by: (a) carotid-cardiac baroreflex testing; (b) Valsalva testing; and (c) power spectral determinations of beat-to-beat R-R intervals and arterial pressures.

Two secondary objectives of this study were: (1) to describe and compare beat-to-beat R-R interval and arterial pressure responses to Valsalva maneuvers obtained during microgravity to those obtained during hypergravity

and normogravity; and (2) to determine if salivary amylase level is a useful marker for predicting susceptibility to gravitationally-induced motion sickness and/or orthostatic intolerance.

Sixteen unmedicated test subjects completed our 40-parabola KC-135 protocol. Of these sixteen subjects, ten were susceptible to varying degrees of motion sickness and six were not. Of the ten susceptible subjects, six had emesis during and/or after parabolic flight and four did not. Of the six subjects who had emesis, three were "very susceptible" to motion sickness (i.e., repeated bouts of emesis throughout most of the flight) and three were "moderately susceptible" (i.e., only one or two bouts of emesis toward the end of flight). "Mildly susceptible" subjects were the four subjects who had prodromal symptoms but who did not vomit at any time during or after flight. These subjects were nonetheless differentiated from the six completely resistant subjects, whose Graybiel motion sickness scores never exceeded 0-1 at any point during or after parabolic flight.

Baseline salivary amylase levels were measured in all sixteen subjects (preflight). Mean levels for each of the motion sickness susceptibility groups outlined above were as follows: (1) "Very susceptible" group (n = 3): 337,433 U/L; (2) "Moderately susceptible" group (n = 3): 178,080 U/L; (3) "Mildly susceptible" group (n = 4): 105,275 U/L; and (4) "Resistant" group (n = 6): 114,167 U/L. Thus, baseline salivary amylase levels are higher, on average, in individuals most susceptible to parabolic flight-induced motion sickness. These preliminary findings are similar to the findings of Gordon, et al., for subjects experiencing either a seasickness stimulus or a cross-coupled (Coriolis) stimulus.

Of the sixteen subjects studied, two females were not able to complete 30-min tilt tests *preflight* due to typical vasovagal reactions. The other 14 subjects (ten males, four females) finished 30-min preflight tilt tests without symptoms.

Postflight, five of the sixteen subjects were not able to complete 30-min tilt tests, and three additional subjects experienced symptoms while managing to finish. The remaining eight subjects were asymptomatic, finishing their postflight tilt tests uneventfully.

Of the five subjects with frank orthostatic intolerance postflight, three subgroups were identified based upon the pattern of intolerance observed: (1) tilt-intolerant group 1, consisting of two females who had hypotensive vasovagal reactions. The postflight vasovagal reactions of one of these females was similar to (but relatively earlier than) the vasovagal reaction she experienced preflight; (2) tilt-intolerant group two, consisting of one male subject who developed significant symptomatic orthostatic tachycardia postflight (clinically analogous to a patient with the "postural orthostatic tachycardia syndrome," or POTS, and accompanied by mild orthostatic hypertension rather than hypotension); and (3) tilt-intolerant group 3, consisting of two males who had what we would term "prostration" - i.e., extreme discomfort in the upright position necessitating tilt-back, sometimes without a clear precipitating hypotensive event, but often preceded by falls in middle cerebral arterial blood flow velocity as measured by transcranial Doppler. [Note that the "prostration" type of intolerance has also been described by Buckey, et al. for two astronauts post-spaceflight (Orthostatic intolerance after spaceflight. *J. Appl. Physiol.* 81 (1):7-18, 1996), although transcranial Doppler measurements were not performed in that investigation.]

Interestingly, the two subjects in the "prostration" group above both had emesis during and/or after parabolic flight, whereas the "POTS-like" subject in tilt-intolerant group 2 above did not have emesis. Of the two females with postflight vasovagal syncope (group one above), one had emesis during parabolic flight while one did not.

Of the three subjects who had symptoms during postflight tilt testing but who managed to finish the 30-min test, one (a motion-sickness resistant female) had symptoms (lightheadedness) only toward the end of the test, when her hemodynamic pattern began to deteriorate in a fashion similar to that of the male POTS-like subject in tilt-intolerant group 2 above. The other two symptomatic finishers (both vomiters during parabolic flight) verbalized but tolerated intermittent symptoms similar to those expressed by the frankly intolerant "prostration" group above (i.e., nausea and lightheadedness sometimes unaccompanied by changes in vital signs). The

postflight tilt test of one of these latter "symptomatic finishers" was particularly instructive. This subject refused the relief of tilt-back despite an episode of emesis in the upright position. Her upright episode of emesis was preceded by nausea, which, as it progressed, became accompanied by the following hemodynamic changes: (1) relative tachycardia; (2) relative hypotension; (3) falls in stroke volume and total peripheral resistance; and, (4) falls in middle cerebral arterial blood flow velocity as measured by transcranial Doppler. Interestingly, this subject's nausea reached a crescendo of emesis just prior to the time that medical monitoring dictated that her tilt test be terminated on hemodynamic grounds. Following the Valsalva-like retching activity of emesis, there was a *resetting* of her vital signs and a return to stable, upright, pre-nausea hemodynamics. These findings suggest that signals from the vestibular receptors (which are necessary for motion sickness induction) may contribute to the regulation of upright hemodynamics in man, possibly mediated via brainstem areas that influence both cardiovascular and visceral responses to nauseogenic stimuli. Findings also suggest that the physical act of vomiting during severe motion sickness may actually be hemodynamically *protective*, since the emetic reflex appeared to ameliorate an impending exhaustion of the sympathetic nervous system associated with ever-increasing levels of nausea.

We have tentatively concluded that the following factors may be associated with an increased risk for deficits in orthostatic tolerance post-parabolic flight compared to pre-parabolic flight:

1) *Female gender*: Four of the six female subjects (66%) had lessened orthostatic tolerance postflight compared to preflight, in contrast to three of ten males (30%). Of the two female subjects who did not experience a pre- to postflight deterioration in tilt-test performance, one was completely stable and asymptomatic during *both* the pre- and postflight tilt tests, while the other had a vasovagal reaction preflight but not postflight (postflight, this final female subject was a "symptomatic finisher of the prostration type").

2) *Motion sickness*: Four of the six subjects who vomited during parabolic flight (66%) experienced a pre- to postflight deterioration in tilt-test performance, in contrast to three of ten subjects (30%) who did not vomit. Of the two vomiters who did not experience a pre- to postflight deterioration in tilt-test performance, one (a male "moderately susceptible" to motion sickness) was completely free of motion sickness symptoms by the time of his postflight tilt-test. The other (a female "very susceptible" to motion sickness) was the same subject mentioned at the end of the "female gender" risk factor section above, who had a vasovagal reaction to tilt *preflight* but who finished tilt (symptomatically) postflight. Of the ten subjects who had *any degree* of motion sickness during flight, five (50%) had a deterioration in tilt-test performance postflight, compared to two of six subjects (33%) in the group completely resistant to motion sickness. Overall, these findings suggest that while motion sickness and emesis-related fluid loss commonly accompany post-parabolic flight orthostatic intolerance, these conditions are not sufficient to explain intolerance in certain individuals.

3) *Higher than average basal salivary amylase level*: The mean basal salivary amylase level for the entire group ($n = 16$) was 162,588 U/L. The mean basal salivary amylase level for the seven subjects who experienced a pre- to postflight deterioration in tilt-test performance was 227,529 U/L. The mean basal salivary amylase level for the nine subjects who did *not* experience a pre- to postflight deterioration in tilt-test performance was 112,078 U/L. Thus, like the amylase data presented earlier with respect to motion sickness susceptibility, higher mean basal salivary amylase levels were also found in individuals most susceptible to postflight deteriorations in tilt test performance.

4) *First time flyer*: Of the seven subjects who experienced a pre- to postflight deterioration in tilt-test performance, four (57%) had no prior parabolic or acrobatic flight experience. Of the nine subjects who did not experience a pre- to postflight deterioration in tilt-test performance, only two (22%) had no prior parabolic or acrobatic flight experience.

More detailed statistical analyses of the above data are in progress. Statistical analyses of data related to the effects of motion sickness on Valsalva responses, carotid-cardiac baroreflex responses, and heart rate variability are also in progress. Power spectral density analyses of the R-R intervals of some subjects (both motion sick and non-motion sick) have been performed. R-R interval data were collected during controlled frequency

breathing prior to takeoff and immediately after landing in both the supine and seated positions. In subjects with recent emesis, supine measurements of total power (0.0-0.3 Hz), high frequency power (0.2-0.3 Hz), low frequency power (0.05-0.15 Hz), and very-low frequency power (< 0.05 Hz) have all tended to increase postflight (i.e., immediately after emetic episodes) versus preflight. However, the rises in high frequency power after emesis are proportionately much greater than the rises in low frequency power, suggesting a relative increase in parasympathetic modulation. In subjects insusceptible to emesis, on the other hand, *declines* in total power, high frequency power, and very low frequency power are more common postflight (vs preflight), accompanied by *increases* in low frequency power. These increases in low frequency power and decreases in high frequency power result in a higher "low-to-high frequency ratio" postflight in emesis-resistant subjects, suggesting a relative increase in *sympathetic* modulation.

Data pertinent to the effects of acute microgravity and hypergravity on autonomic cardiovascular responses to the Valsalva maneuver have been analyzed, and preliminary results were presented at the 12th Man in Space Symposium in Washington, DC, June 8 - 13, 1997 (abstract referenced below). A corresponding manuscript has been completed with intended submission to the *Journal of Applied Physiology*.

Earth benefits of this overall research include an enhancement of our understanding of the role that the vestibular apparatus plays in regulating the human cardiovascular system, particularly as it relates to orthostatic tolerance. Information gained from this research may prove useful for the development of new therapeutics for both syncope and motion sickness.

FY97 Publications, Presentations, and Other Accomplishments:

Schlegel, T.T., Benavides, E.W., Barker, D.C., Brown, T.E., Harm, D.L., and Low, P.A. "Cardiovascular and valsalva responses during parabolic flight." (Abstract) 12th Man in Space Symposium, Washington, DC (June, 1997).

Vestibular-Autonomic Interactions During and After Prolonged Gravitational Changes

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Post-Doctoral Associates: 0

Responsible NASA Center: Johnson Space Center

Task Description:

This project addresses two of the recent emphases of NASA's Space Physiology and Countermeasures Program; namely (1) to utilize a ground-based study to address the effects of vestibular-autonomic interaction as related to post-space flight orthostatic intolerance, and (2) to determine basic mechanisms of physiologic responses to hypergravity using human subjects.

To accomplish these goals, long-duration centrifugation (+3Gx and +3Gz) is being used as a stimulus to generate vestibular-mediated responses in humans that are subject to interindividual variability (i.e., upbeating or "Lz" nystagmus slow-phase velocity responses during +Gz centrifugation, posture-platform performance changes after centrifugation, and other vestibular-mediated responses). Changes in responses to various provocative autonomic *cardiovascular* stimuli (including Valsava maneuvers, carotid-cardiac baroreflex studies, heart rate variability tests, and head-up tilt) after the onset of vestibular-mediated changes in subjects most susceptible to such changes are then being compared to the autonomic or cardiovascular changes that occur, if any, in subjects less susceptible to vestibular-mediated changes who experience the same centrifugation stimulus.

Central hypotheses are that subjects manifesting the greatest changes in vestibular function during and/or after centrifugation will also exhibit the most significant deficits in autonomic cardiovascular control and orthostatic tolerance. Confirmation or disconfirmation of these hypotheses should enhance the existing understanding of the etiology of post-space flight orthostatic intolerance, potentially leading to the development of more optimal (i.e., mechanism-centered) countermeasures.

Data collection for this study has just begun (July-August, 1997). Thus far, eight subjects have ridden the NAMRL centrifuge (Pensacola, Florida) for 30 min in +3Gx, and for lesser periods of time in +3Gz. Pre-, in-, and post-centrifugation neurovestibular and cardiovascular data are preliminary and are currently being analyzed.

Additional long-duration centrifugation sessions in +3Gz (G-suited) are scheduled for October-November, 1997.

Earth benefits of this research include an enhancement of our understanding of the role that the vestibular apparatus plays in regulating the human cardiovascular system, particularly as it relates to orthostatic tolerance. Information gained from the research may prove useful for the development of new therapeutics for both sycops and motion sickness.

Effects of Hindlimb Suspension on Skeletal Muscle Growth

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No Co-Is Assigned to this Task

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Students Funded Under Research: 3

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Task Description:

The process of growth and regeneration of skeletal muscle are each dependent upon the proliferation of satellite cells. Hindlimb suspension (HS) has been shown to dramatically alter the proliferative activity of satellite cells. In growing muscles, satellite cells exhibit a reduction in their proliferative rate within 24 hours of initiation of unweighting. Proliferation continues to decrease until three days after HS when all mitotic divisions are abolished in the soleus muscle. After a complete cessation of divisions that lasts through five days, a low level of divisions resumes. Proliferations appear to remain suppressed for as long as the limb is non-weightbearing in muscle such as the soleus although in non-antigravity muscles such as the extensor digitorum longus (EDL) proliferations may return to near-control levels after extended periods. It is not known whether satellite cell proliferations will exhibit any compensatory increase when weightbearing (WB) is resumed after a period of HS. In this research, we investigate the ability of myofibers to compensate for the deficit in the number of myonuclei produced during a HS period after WB is reinitiated. The response of satellite cells to HS is not completely suppressed in injured muscles suggesting the mechanisms that control satellite cell proliferations during growth may not be shared in common with those controlling proliferations during regeneration. Although myofibers play a role in regulation of satellite cell proliferative activity in intact, growing muscles, during the early phases of regeneration response, all fibers have been destroyed. After the formation of new fibers in the regenerate, regulation may again come under the control of the myofibers. Preliminary studies in our lab suggest that regeneration in a HS environment is reduced when compared to WB controls. In this proposal we will determine where along the continuum of the regeneration response that the HS environment exerts an influence. The regeneration response is originally broken down into two phases: 1) from the start of regeneration to the time when nascent myofibers are being innervated, and 2) from the time of innervation to the completion of regeneration. The period before innervation is characterized by the proliferation and fusion of satellite cells to form nascent myofibers. The second phase is characterized by the growth and development of new myofibers. The regulation of satellite cell proliferative activity during Phase I is specific to regeneration whereas regulation during Phase II is common to regeneration and growth. In this manner we hope to determine if the environment of unweighting is selective to only a portion of the regeneration response or whether all aspects of satellite cell proliferative activity are altered without respect to the manner in which they are regulated. The results of these studies will determine the direction taken in subsequent experiments to understand the

mechanism whereby unweighting alters muscle development and develop countermeasures to the deleterious effects of development.

The time course in the expression of MRFs during regeneration was studied using *in situ* hybridization and Northern analysis. The intervals after injection of the myotoxic venom Notexin into the soleus muscle were 3, 10, 14, 24, and 28 days. The plot from density scanning of autoradiographs shows the percentage change from control of expression of Myogenin (MYG) MyoD (MYOD), Myf5 and MEF2. Results were normalized with glyceraldehyde-6-phosphate dehydrogenase (GAPDH). MyoD and myogenin increased to maximum expression at 3 days. MyoD returned to near control levels by 10 days and myogenin by 28 days. MEF2C exhibited a delay that did not start until 3 days and remained elevated through 28 days. Northern analysis was able to show that subtle differences are present in the expression pattern of MRFs in oxidative compared to glycolytic muscles. We are now in a position to carry out experiments to compare expression patterns in muscles regenerated under conditions of HS.

A second series of experiments was carried out to investigate the influence of innervation on the expression of MRFs during regeneration. We found that if the muscles are denervated at the time they are injected with Notexin, MRF expression was changed. The major change was in the expression of Myf5, which was now expressed at very high levels starting at 3 days. MEF2C expression showed a inverse level of expression to myogenin in both cases. When the muscle was simply denervated, expression of all MRFs increased

We find these results very interesting because of the known changes induced by HS in the nerve-muscle axis. We have also examined the expression of MRFs myogenin and MyoD in growing soleus muscles in WB and HS animals. With *in situ* hybridization we found major expression in a focal punctate pattern located at the periphery of the myofibers. Based on the results of labeling studies in the lab, we have tentatively concluded that expression of MyoD and myogenin is mainly associated with satellite cells in the muscle. When the animals are placed in HS the punctate expression of MRFs is lost as the cells stop dividing and increases as the cells resume mitotic activity. Expression over the myofibers is less, and myogenin shows more than MyoD. However, Northern analysis provided no significant difference in the expression of MyoD or myogenin during HS. These studies have shown that MRF expression will not be a suitable marker to monitor effects of HS on skeletal muscle. They appear to have potential to be used as markers for events during muscle regeneration with and without HS.

The relationship of myogenic transcription factors to muscle mass

Myogenic regulatory factors myogenin and MyoD are expressed at low levels in mature muscles. Myogenin is the dominant factor expressed in slow muscles whereas MyoD is the dominant factor expressed in fast muscles. The factors in each of the respective types of muscles is thought to play a role in the stability of the phenotype in these muscles. We investigated the effects of different treatments that alter myofiber phenotype and mass on the expression of myogenin and MyoD. Using clenbuteral and triiodothyronine in combination with overload, we were able to induce a slow to fast transformation of myosin phenotype with or without an increase in mass, or an increase in mass without a transformation of myosin phenotype. By separating each of these variables and using Northern analysis, we were able to determine that MyoD and myogenin expression are more closely related to myofiber myosin phenotype than muscle mass. In parallel experiments we also demonstrated that during hindlimb suspension, as the muscle atrophies and exhibits a slow to fast myofiber type transition, there is an upregulation of MyoD (determined by semi-quantitative PCR). Our hope was that if MyoD or myogenin exhibited a change in expression related to mass, they could have been used as markers of mass changes in the muscles during experimental treatments designed to inhibit atrophy or induce the fibers to grow. We have shown that changes in the expression of these factors are reliable indicators of myosin phenotypic changes. The absence of any correlation of MyoD or myogenin expression during atrophy suggests that other factors may be involved. We have submitted a manuscript describing these studies.

Role of DNA unit size and number in skeletal muscle growth

A myofiber can be theoretically subdivided into a series of DNA units. A DNA unit is regarded as a myonucleus and the volume of cytoplasm that immediately surrounds it. Myofibers grow by increasing the number of DNA

units and the size of each unit. The size of the unit is restricted to a maximum volume, mostly by the limits of diffusion of mRNAs. We examined the relative contribution of DNA unit size and number to skeletal muscle growth. The very rapidly growing turkey pectoralis major muscle was used as a model for study. Labeling studies using BrdU had demonstrated previously that pectoralis muscle growth can be operationally subdivided into two phases. The first phase (0-9 weeks) is characterized by satellite cell divisions and fusions (increase in DNA unit number), whereas growth beyond 9 weeks is dependent on increase in the size of the recruited units (Mozdziak et al., 1994). To further study the potential of DNA unit size to support muscle growth, the pectoralis major of young poults was irradiated at a dose known in other systems to "sterilize" the muscle of satellite cells. Using this model, we asked how growth potential of the muscle would be altered if myonuclear increase was greatly reduced and growth became almost totally dependent on increase in DNA unit size. We hypothesized that the muscle would compensate by increasing DNA unit size and/or protracting the phase of myonuclear accretion. We found that a single acute reduction in the production of myonuclei (as a result of killing satellite cells through irradiation) resulted in a permanent retardation in the growth of the irradiated muscles. DNA unit size did not increase beyond control values suggesting that each unit attains maximal size during normal growth. Based upon these studies we concluded that myonuclear accretion was the major determinant of muscle growth. However, a major observation that came from these studies was that the muscle did not recover from the reduced myonuclear accretion that occurred during a finite duration during the growth period. The deficit remained through maturity.

Previous studies in other labs concluded that irradiation killed all satellite cells in a muscle, based on the observation that irradiated muscles exhibited little growth or hypertrophy. They did not directly determine that satellite cells were no longer present following irradiation. Our studies, showing growth retardation post-irradiation, initially supported the hypothesis of a complete loss of satellite cells. However, we successfully isolated a viable population of satellite cells from the irradiated muscles. In fact, satellite cells irradiated *in vivo* or *in vitro* had growth and fusion kinetics the same as the non-irradiated controls (Mozdziak, et al., 1996), suggesting that killing the satellite cells was not the reason why the irradiated muscles did not grow. Rather they strongly suggest that during the growth period following irradiation, remaining healthy satellite cells were not recruited to add additional myonuclei to the muscle fibers, even though growth *in vitro* indicated they had sufficient proliferative potential. The reason for the inability of the muscles to recruit the potential of the remaining satellite cells remains unknown. It is possible that the mechanisms utilized to induce mitotic divisions and subsequent fusions of satellite cells during growth are no longer available to the muscle as maturity is reached and, as a result, growth is retarded. We have now extended these observations to show that HS of even short duration produces a lasting growth retardation of the antigravity soleus muscle. In both paradigms, a reduction in myonuclear accretion during a critical period of growth results in a loss of growth potential.

In summary, these studies collectively support the conclusion that the major determinant of muscle growth is increase in DNA. Although increased DNA unit volume is a component of later stages of growth, we found no compensatory changes in unit size when muscle growth was retarded. When a muscle is growth-retarded, there is limited or no recruitment of available satellite cells to increase myonuclei (DNA units). The results of these studies have led to the working hypothesis that there are no mechanisms of compensatory growth in the satellite cell/myofiber axis when myonuclear accretion is reduced during the growth period. These studies add further support to our overall hypothesis that growing muscle, when subjected to extended periods of weightlessness, will exhibit a growth retardation that will persist even when weightbearing is reinitiated.

We are just now beginning to obtain a better understanding of the effects of weightlessness on growing and adult skeletal muscle. The work being carried out may provide a means to modulate the growth process in a way that will prevent the retardation that occurs during prolonged periods of non-weightbearing and to induce compensatory growth in muscles that have not reached their full developmental or functional potential. Likewise, the results of these studies may also provide a means to prevent or attenuate the atrophy that occurs in adult muscles during periods of disuse.

FY97 Publications, Presentations, and Other Accomplishments:

Mozdziak, P.E., Greaser, M.L., and Schultz, E. "Myogenin and MyoD expression are more closely related to myosin heavy chain composition than muscle mass." (Abstract) *J. Animal Sci.*, 75, 121 (1997).

Mozdziak, P.E., Schultz, E., and Cassens, R.G. "Myonuclear accretion is a major determinant of avian skeletal muscle growth." *Am. J. Physiol.*, 272, C565-571 (1997).

Ulibarri, J.A., Best, T.A., Mozdziak, P.E., Schultz, E., Case, M., and Vanderby, R. "Nitric oxide stimulates myoblast proliferation *in vitro*." (Abstract) *Med. Sci. Sports Exercise* (1997).

Prevention of Bed Rest Osteoporosis: Resistive Exercise

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Task Description:

In bed rest and space flight, bone losses occur primarily in the spine and lower body regions, with no changes in the upper extremities. This suggests that those regions with the greatest decrease in load compared to pre-flight or pre-bed rest lose the most bone. This loss of skeletal mass may prove hazardous to astronauts on flights of long duration, not only because hypercalciuria might lead to the formation of renal calculi during flight, but axial skeletal fractures may occur upon return to Earth's gravity. Several studies of resistive exercise indicate that bone density increases with training programs employing low repetition, high load weight lifting. Using ambulatory subjects to test a 12-week resistive exercise regimen, similar to the exercise we propose for this bed rest study, we found significant increases in lumbar, thoracic, and total spine bone mineral density.

A resistive exercise program nearly identical to the one we propose here was tested during 17 weeks of bed rest on one male subject. In this pilot study, calcium balance became positive after 10 weeks of bed rest. While the hip and heel showed some bone loss (2.2% and 4%, respectively), these losses were approximately half of those in the control group (4% and 10%). Lumbar spine bone mineral density increased 1.5% in the exercise bed rest subject, whereas spine BMD in the controls decreased by 3.4%. The exercise protocol preserved both leg muscle mass and muscle strength; control subjects lost significant lower body muscle mass and strength. Finally, the exercise subject showed increases in markers of bone resorption, but more importantly, he experienced comparable increases in markers of bone formation. Control subjects experienced similar increases in markers of bone resorption, while markers of bone formation did not change significantly.

From these studies, it appears likely that an in-flight program of resistive exercise could counteract the musculoskeletal effects of disuse. Resistive exercise offers the advantage of directly preventing the decreased bone strain occurring in disuse and provides a regional countermeasure for the local bone loss. While the ambulatory and pilot study results appear promising, further validation of the exercise protocol must be

conducted before implementing a similar resistive exercise protocol in space. The exercise countermeasure must be validated on a larger subject population in order to show statistical significance of the results, and the study should include both men and women. We will determine the effectiveness of intense resistive exercise as a countermeasure to bone and muscle loss in a 17-week bed rest study involving twenty bed rest subjects (ten exercise subjects and ten controls). We will employ a Horizontal Exercise Machine and a modified version of the exercise protocol employed in the above-mentioned ambulatory study and pilot bed rest study. We will obtain pre-bed rest measures of calcium balance, bone density, metabolic bone markers, lean body mass, muscle strength, and muscle volume, and compare these with measurements obtained during early, mid, and late bed rest. These results will also be compared to results from our previous 17-week bed rest studies conducted without countermeasures.

The NASA Baylor Methodist bed rest facility was established through renovation of an area at Methodist Hospital. Nursing staff were hired to tend the facility constantly. A study coordinator and physical trainer were also hired to staff the facility.

Post menopausal and senile osteoporosis are major health care problems in the US. The financial burden of osteoporosis to our health care system is expected to increase with the increasing number of geriatric population in the US. Hormonal replacement and bisphosphonate are two approaches to treating this disorder, but both have adverse side effects that may prevent their usage for certain individuals. An effective means to control the health care costs of osteoporosis would be to prevent osteoporosis. Literature abounds with descriptions of bone response to different exercise regimens, with sometimes contradictory reports for the same exercise program. Sources of variable bone response to exercise are the physical condition and hormonal status of the population studied, individual diet, and the activities of daily living of the individuals.

This bed rest study isolates the exercise protocol from other activity, controls diet, and utilizes a population with normal health and hormonal status. Maintenance of bone density in the bed rest volunteers will indicate that the intense resistive exercises tested are effective in preventing bone loss and would be appropriate for individuals wanting to maintain bone density as they age. The intensity of the program is designed so that the exercise program can be accomplished within a limited time frame of less than 1.5 hours per day because of space flight schedule constraints. This time constraint is also appropriate to the busy schedules of many individuals in modern society. Information from this study will be available for dissemination to the population at risk and to health care professionals to guide osteoporosis prevention and treatment programs.

Modeling of Cardiovascular Response to Weightlessness

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Post-Doctoral Associates: 0

Task Description:

Results obtained by the investigators in ground-based experiments and tests aboard the NASA KC-135 with a hydraulic simulator of the cardiovascular system have confirmed that a simple lack of hydrostatic pressure within an artificial ventricle causes a decrease in stroke volume of 15-20%. These results are in basic agreement with echocardiographic experiments on STS-51 D, which documented a 15% decrease following a three-day period of adaptation to weightlessness. The hydrostatic environment of the cardiovascular system, however, is much more complicated than that modeled in any computer models or *in vitro* experiments to date. One can reason that fluid shifts from the lower body to the thorax serve to increase right atrial pressure and boost cardiac output (CO). The concurrent release of gravitational force on the rib cage tends to increase chest girth and decrease pericardial pressure, augmenting ventricular filling. The lack of gravity on pulmonary tissue allows an upward shifting of lung mass, causing a further decrease in pericardial pressure and increased CO. Additional effects include diuresis early in the flight, interstitial fluid shifts, gradual spinal extension and movement of abdominal mass, and redistribution of circulatory impedance because of venous distention in the upper body and the collapse of veins in the lower body. While neurohumoral regulation of flow and pressure presents an additional dimension of complexity, it is the hypothesis of this work that the simple lack of hydrostatic pressure in microgravity generates several purely physical reactions that underlie and may explain, in part, the cardiovascular response to weightlessness. The problem was studied by developing a numerical model incorporating important physiological fluid and structural elements sensitive to hydrostatic pressure, while maintaining authentic compartmental and overall systemic impedance. An analogous physical model was built for testing in various postures in 1-G and in microgravity and hypergravity aboard the KC-135. Results will be compared to available *in vivo* measurements. Development of the numerical model to date has involved modifying a model of the entire circulation to include short-term effects of hydrostatic pressure in the ventricle and venous system to predict changes in cardiac performance and regional fluid shifting in response to changes in gravity in different postures. Future plans include improving the model and incorporating additional short-term effects, such as thorax/abdomen structural effects on atrial and ventricular transmural pressure, as well as intermediate and long-term effects of fluid volume adjustment, extravascular fluid shifts, spinal extension, and some aspects of neurohumoral control. The physical model has been systematically expanded and improved to incorporate right and left ventricles and three regions of the systemic circulation and the pulmonary circulation as well as the effects of thoracic pressure on ventricular filling. (The physical model can incorporate only short-term responses because of the limited duration of zero-G aboard the KC-135.) Results from a second, more extensive, computer

model of the systemic arteries were used to guide the design of resistance and compliance elements for a realistic, but efficient physical model. Both models will be used to predict and assess the efficacy of measures to accelerate cardiovascular adaptation to microgravity and the efficacy of countermeasures to post-flight orthostatic intolerance including preflight dehydration, lower body negative pressure, and pre-landing fluid loading. Both models will provide platforms for evaluating further ideas for improved human performance and safety in space.

Development continued this year on a physical model of the human cardiovascular system flown aboard the NASA KC-135 in zero-gravity and on a computer model simulating the effect of gravity on the cardiovascular system.

The life-size physical model incorporated four regional mock circulation units representing the cranial, central and caudal systemic circulation, and the pulmonary circulation (new for this year). Each mock circulation unit contained proximal and distal resistors and proximal and distal compliance elements. Resistance and compliance values were matched to physiologic values. The mock circulation units were redesigned with new flow resistors utilizing compressed open-cell foam. Resistance values were variable by adjusting the amount of compression of the foam. A prototype of the new mock circulation unit was flown aboard the KC-135 in June 1997. The new resistor design proved much more controllable and reliable than the previous one, which used a sliding plate to adjust the flow area through a porous block.

A new peripheral venous pool monitored with both pressure and displacement (correlated to volume) sensors was added to the experiment to improve resolution of venous pool volume across the range of pressure in which small changes in pressure correspond to large changes in volume. An artificial right ventricle was also added to pump fluid through the pulmonary circulation.

The physical model was flown aboard the KC-135 in September/October 1997. The experiment was monitored with an accelerometer, five ultrasonic flow transducers (one for each region plus one for left ventricular output), nine displacement transducers (one on each compliance chamber plus one on the peripheral venous pool), and ten pressure transducers (one at the input to each region, one at the left ventricular output, one in each atrium, one in the peripheral venous pool, and two in the left ventricle). Data was collected in three postures - upright, supine, and launch - at several cardiac flow rates with physiologic distribution of flow to the regions. Analysis of the data is in progress, but initial observations showed a decrease in cardiac output during 0-G compared to 2-G and shifting of fluid away from the caudal region and the peripheral venous pool toward the cranial region in the upright posture. Fluid shifted toward the venous pool in 0-G in the launch posture. Little fluid shifting or changes in cardiac flow were evident in the supine posture.

The numerical model, originally written for clinical diagnostic purposes, was modified this year to simulate the effect of gravity on the cardiovascular system. Systolic contraction of the left ventricle drives the blood flow. A time-dependent elastance curve defines ventricular behavior during systole, while passive filling incorporating a hydrostatic pressure term defines diastole. Uni-directional valves prevent backflow into the ventricle from the aorta.

The code features a branched arterial tree with 28 arterial segments in the systemic circulation. In each arterial segment, 2-14 nodes are defined at which pressure, velocity, and cross-sectional area are calculated. The terminal arterial segment at the distal end of each branch is a modified windkessel. The viscoelastic nature of the arterial wall and wall shear due to pulsatile flow are included to account for damping mechanisms that create waveforms seen physiologically.

A lumped parameter model of the venous circulation, right heart, and pulmonary circulation completes the circulation loop. The venous circulation was recently divided into three regions - cranial, central, and caudal - in order to track regional fluid shifting. A hydrostatic term was included in the pressure calculations for each region. Currently, venous compliance is modeled by a linear approximation, but will soon be modeled by a three segment relationship dependent upon pressure.

With user-defined inputs, the code produces a steady-state waveform for pressures, volumetric flow rates, and velocities at any defined location in the circulation. The numeric model confirmed that a loss of hydrostatic pressure in the right and left ventricles significantly decreases stroke volume by about one third (1-G versus 0-G in upright posture). Average ventricular pressures and volumes decreased by a similar amount. The changes in stroke volume and regional fluid shifts for the cranial, central, and caudal regions will be quantified for the postures of upright, supine, launch, and sitting.

The potential applications and advancement of the numeric code are many. A more detailed venous and pulmonary circulation could be easily implemented as a branched distributed model. Effects of variation in thoracic pressure and its connection with central venous pressure could be easily investigated since a thoracic pressure term is included in the lumped parameter circulation. The inclusion of simulated left and right atria could improve results. Currently, the model produces a steady solution to defined inputs. A baroreceptor reflex, written for the code, but not currently implemented, will allow long-term transient response to be pursued. Addition of transvascular fluid shifts may be important in modeling long-term response. We would also like to use the model to more directly predict orthostatic intolerance by monitoring cranial arterial pressure and comparing it to a minimum level below which fainting would occur. Also, gravitational terms are not currently included in the arterial side of the circulation, but may add a small but noticeable fluid shift due to gravity and posture changes.

The findings from these models lend support to the notion that the purely biomechanical response to weightlessness, *i.e.*, the direct response of the heart, blood and blood vessels to the elimination of hydrostatic pressure, motivates the many other changes in the cardiovascular system observed in astronauts. In these models, changes in cardiac filling and flow and vascular pressures and fluid shifting have been documented in the absence of all controls and adaptation mechanisms, including neurohumoral influences. The role of these phenomena in causing subsequent changes in circulating fluid volume, blood chemical and cellular composition, and cardiac and vascular properties, including peripheral resistance, remains to be investigated.

Hypotension and tachycardia are severe for many astronauts. Approximately half cannot tolerate a 10-minute stand test immediately after landing. Post-flight orthostatic intolerance first appeared after the fourth manned Mercury flight of only 34 hours and has occurred after flights of just nine hours. Most nonastronauts have experienced orthostatic intolerance at one time or another and for some people the effects are chronic and debilitating. While long-term adaptations to microgravity may contribute to reduced tolerance, it is clear from the above results and from patients on Earth that short- to intermediate-term effects must play an important role. Increased leg compliance, increased capillary permeability, deteriorated baroreceptor response, and hypovolemia are some of the causes that have been forwarded. The partial success of pre-landing ingestion of saline in preventing orthostatic intolerance indicates that hypovolemia is at least partially responsible; however, these results do not preclude the contributory effects of other factors. This project focuses on the effect of changes in hydrostatic pressure on the cardiovascular system, an effect that is present not only in launch and landing for astronauts, but also during changes in posture for people on Earth. Further study of this mechanism may lead to more effective countermeasures for all sufferers of orthostatic intolerance.

FY97 Publications, Presentations, and Other Accomplishments:

Pantalos, G.M., Mathias, J., Sharp, M.K., Watenpaugh, D., Buckey, J., Parnis, S., Hargens, A., and Thornton, W. "Variations in esophageal and abdominal pressure in humans during parabolic flight." American Society for Gravitational and Space Biology Conference, Charlotte, NC (October 23 - 26, 1996).

Sharp, M.K., Pantalos, G.M., Gillars, K.J., O'Leary, D.S., and Woodruff, S.J. "Modeling of cardiovascular response to weightlessness." ASME Summer Bioengineering Conference, Sun River, OR (June 11 - 15, 1997).

Woodruff, S.J., Sharp, M.K., and Pantalos, G.M. "Compact compliance chamber design for the study of cardiac performance in microgravity." ASAIO J., 1, 316-320 (1997).

Effects of Bedrest on Forearm Muscle Reflexes

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No Co-Is Assigned to this Task

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Students Funded Under Research: 13

Post-Doctoral Associates: 3

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Task Description:

The overall objective of this project is to examine the effects of two weeks of bedrest on the sympathetic nerve responses to rhythmic and static forearm exercise. We postulate that sympathetic nerve responses will be increased because lactic acid production and forearm interstitial volume will be increased. We further postulate that forearm handgrip exercise and/or intermittent forearm compression will act as countermeasures to obviate the effects of bedrest.

The grant was initially funded in March of 1995. We began our first group of studies in November of 1995. To date we have completed and written a manuscript for the *Journal of Applied Physiology*. In this report we demonstrate that resting sympathetic nerve activity is diminished after 2 weeks of head down bedrest (HDBR). In a prior report we demonstrated that limb immobilization reduced peak forearm blood flow (Silber et al., Reversible impairment of forearm vasodilation after forearm casting. *J. Appl. Physiol.* 1990; 68(5): 1945-1949). Accordingly, we have become interested in determining whether bed rest would reduce peak forearm blood flow. We have completed a group of experiments (an abstract is being presented at the Canadian Society of Exercise Physiology Conference and a manuscript of this work is in progress) in which we examined the effects of two weeks of HDBR on peak forearm blood flow. These experiments demonstrated that the reactive hyperemic blood flow response was reduced after bed rest. Additionally, we found that responses to a cold pressor maneuver were also decreased in the human forearm after bed rest. These findings suggest that both dilator and constrictor mechanisms are attenuated after bedrest and by inference space flight.

We are now beginning experiments to examine sympathetic responses to tilt before and after bedrest.

The initial reason for performing these experiments was to gain insight into the effects of prolonged space flight on muscle reflexes. We postulated that the increase in interstitial volume and the potential changes in muscle fiber types would lead to a predilection towards heightened sympathetic responses to exercise. Additionally, we speculated that the muscle changes described above could contribute to the heightened sense of forearm fatigue sometimes mentioned by astronauts during EVAs.

It is important to emphasize that bedrest and the accompanying autonomic changes seen are a common accompaniment of many major disease processes. Accordingly, the study of autonomic control after bedrest has major implications for these problems. For example, after a number of illnesses patients are placed at bedrest

for a number of days. With the resumption of activity there are a number of important difficulties noted by these patients (e.g., fatigue). The mechanisms for this fatigue are difficult to study in the ill patients. Understanding the ramifications of bedrest have important implications for this issue. Additionally, individuals with cardiovascular disease are often placed at bedrest for a number of days and concurrently receive medication which impairs postural control. An understanding of the vascular and autonomic responses that are due solely to bedrest would also have important implications for our understanding of postural difficulties in patients with severe cardiovascular insults.

FY97 Publications, Presentations, and Other Accomplishments:

Crawford, P., Good, P.A., Gutierrez, E., Feinberg, J.H., Boehmer, J.P., Silber, D.H., and Sinoway, L.I. "The effects of supplemental oxygen on forearm vasodilation in humans." *J. Appl. Physiol.*, 82(5), 1601-1606 (1997).

Leuenberger, U.A., Silber, D.H., Herr, M.D., Gray, K.S., and Sinoway, L.I. "Mechanisms of sympathetic neural activation during rhythmic handgrip exercise in congestive heart failure." (Abstract) *J. Invest. Med.*, 45(3), 219A (1997).

MacLean, D.A., Saltin, B., Rådegran, G., and Sinoway, L. "Femoral arterial injection of adenosine in humans elevates MSNA via central but not peripheral mechanisms." *J. Appl. Physiol.*, 83(4), 1045-1053 (1997).

Mostoufi-Moab, S., Widmaier, E.J., Cornett, J.A., Gray, K., and Sinoway, L.I. "Forearm training reduces the exercise pressor reflex during ischemic rhythmic handgrip." *J. Appl. Physiol.* (In Press).

Potts, J.T., Sinoway, L.I., and Mitchell, J.H. "Afferent mechanisms, medullary sites, and efferent sympathetic responses of the exercise pressor reflex" in "Human Kinetics." (In Press).

Shoemaker, J.K., Pandey, P., Herr, M.D., Silber, D.H., Yang, Q.X., Smith, M., Gray, K., and Sinoway, L.I. "Augmented sympathetic tone alters muscle metabolism during exercise: Lack of metabolic evidence for functional sympatholysis." *J. Appl. Physiol.*, 82(6), 1932-1938 (1997).

Silber, D.H., Leuenberger, U.A., Boehmer, J.P., and Sinoway, L.I. "Flow limitation in CHF patients engages the muscle metaboreflex." (Abstract) *J. Invest. Med.*, 45(3), 219A (1997).

Sinoway, L.I. "Blood flow regulation during exercise in humans with congestive heart failure." ACSM Meeting - Symposium, Denver, CO (May 31, 1997).

Sinoway, L.I. "Effects of space flight on the human body." Palmyra Middle School (8th graders), Palmyra, PA (May 5, 1997).

Sinoway, L.I. "Neural control during exercise: Insights from human experiments." Cornell University, New York, NY (December 16, 1996).

Sinoway, L.I. "Neural control mechanisms during exercise in humans." University of Rochester School of Medicine, Special Cardiology Seminar, Rochester, NY (October 30, 1996).

Sinoway, L.I. "Neural control of the circulation during exercise." Kinesiology Colloquium, Boulder, CO (September 25, 1997).

Sinoway, L.I. "Neural control of the circulation during exercise." Cardiology Research Conference, Denver VA, Denver, CO (September 29, 1997).

Sinoway, L.I. "Neural control of the circulation in heart failure: Insights from human experiments." Kansas State, Manhattan, KS (February 17, 1997).

Sinoway, L.I. "Neural Responses to Exercise in Congestive Heart Failure." University of Nebraska, Omaha, NE (April 18, 1997).

Sinoway, L.I. "The effects of bedrest on neurovascular responses in humans." Kansas State, Manhattan, KS (February 14, 1997).

Sinoway, L.I. "The role of the General Clinical Research Center at Penn State." The Milton S. Hershey Medical Center, Surgical Research Conference, Hershey, PA (November 19, 1996).

Sinoway, L.I. and Mitchell, J.H. "Sympathetic nervous system responses to exercise in heart failure" in "Human Kinetics" (In Press).

Widmaier, E.J., Cornett, J.A., Bodenstein, M., Weaver, M., Sinoway, L.I., and Mostoufi-Moab, S. "Forearm training reduces vascular resistance during ischemic rhythmic forearm exercise." (Abstract) J. Invest. Med., 45(3), 220A (1997).

Microgravity: Sleep Deprivation and Autonomic Control

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$129,788

Task Description:

Astronauts commonly experience difficulty sleeping and are generally sleep deprived. The resultant fatigue may impair physical and mental performance and adversely affect cardiovascular health particularly during stressful conditions. The primary aim of this study is to determine the effects of sleep deprivation (comparable to that experienced by astronauts) on: 1) reflex control of autonomic function, 2) cardiovascular and autonomic responses to stress, 3) forearm exercise endurance, and 4) orthostatic tolerance. Previous studies suggest that syncope is provoked by exaggerated adrenergic stimulation during orthostasis. Thus, a secondary aim is to determine the role of exaggerated adrenergic activation during orthostasis as a mechanism of orthostatic intolerance.

Studies have been completed evaluating the effects of 4 days of sleep restriction (4 hours per night) on autonomic control of the cardiovascular system. These studies suggest that: 1) baseline sympathetic nerve activity, vascular resistance, and blood pressure are not altered by 4 days of sleep restriction; 2) sympathetic neural, vascular resistance, and blood pressure responses to stressors, including orthostasis, exercise, and mental stress, are exaggerated after sleep restriction; and 3) orthostatic tolerance may be impaired in selected subjects, but is not consistently affected by sleep restriction. An ongoing study is designed to evaluate the interactive effects of handgrip exercise and mental tasking on autonomic control of cardiovascular function. Preliminary data suggest that these stressors do not interact mutually to produce an augmented response, but rather interact redundantly to produce a similar response to an individual stress alone.

We are addressing the possible mechanisms of vasovagal syncope. One underlying hypothesis of this project is directed specifically at this question. That is, exaggerated sympathetic neural activation can increase susceptibility to syncope. Both basic science and clinical data are consistent with this hypothesis, and if the results support the hypothesis, it may help guide therapy of individuals at risk for neurally-mediated syncope (both post-flight and of Earth). Another area of interest with possible Earth benefits concerns the effects of sleep deprivation/restriction, since many individuals in the working world are often faced with periods of sleep restriction.

Ultrashort Sleep Strategies During Sustained Performance

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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Efficient management of crew duty and rest time is essential in situations requiring sustained round-the-clock attention and/or activity levels for several consecutive days. Such situations are especially critical in environments where human resources are limited, such as in space flight missions. The disruption of sleep caused by sustained work may result in the operator's reduced alertness and increased risks of error or accidents. Some of the key questions of sleep management are to determine the minimal sleep duration and its optimal placement-distribution within the 24 hrs. In this research project, a sleep management plan is proposed to minimize degradation in performance and to improve safety in crucial operations. The strategy we propose for increasing available operator time is to replace the normal monophasic sleep pattern with a polyphasic (ultrashort) sleep-wake pattern. The hypothesis of this project is that adult humans may have an endogenous ability to adapt to polyphasic sleep-wake patterns and that these may represent feasible, useful strategies for the management of sleep during emergencies or situations of continuous work. Our recent research indicates that polyphasic sleep-wake patterns allow a considerable reduction in total sleep requirements without causing a decrement in performance levels. This study combines theoretical and practical interest: it will increase our understanding of circadian sleep and alertness regulatory mechanisms, and it will also provide tools for developing optimal sleep-wake schedules for sustained performance in space flight missions. This project holds the promise of significant practical application to NASA.

Due to further data analyses on our preliminary studies, we have decided to modify one of the three sleep reduction schedules in this study. In lieu of using the *semi-polyphasic* sleep schedule we had originally proposed (one nocturnal 1.5 hrs plus three diurnal 30 min sleep episodes, see below), we have re-designed this strategy naming it *biphasic*. It consists of one nocturnal plus one diurnal 1.5 hrs sleep episodes, each initiated 12 hrs apart. The biphasic schedule is designed to obtain maximal advantage from the underlying human bimodal circadian (or 12-hr) cycle in sleep/sleepiness propensity. The other two schedules are the *polyphasic* (six 30-min sleep episodes, one very four hours), and *monophasic* (one 3-hr nocturnal sleep episode).

Semi-polyphasic schedules are designed following precise identification of ultradian sleep propensity rhythms which may differ from individual to individual. This may make it potentially more efficient than the biphasic, but also much more complex to tailor to each individual and to use - especially during real emergency

operations. In addition, because of the potential for inter-individual variability, experimentally the semi-polyphasic schedule may lead to increased “noise” in the data, and consequent difficulty in interpreting results. We anticipate that, for these and other reasons, the biphasic schedule may be more efficient, practical and simple to use in the field, and may also provide valuable data for future design of semi-polyphasic schedules, which we expect to evaluate in future studies.

In our proposal, we anticipated studying a total of six subjects during the three year period. Subjects would be studied in pairs (2 at any given time), and each subject would be submitted to each of the three sleep reduction schedules. This involved conducting at least nine 28-day experiments [(6 subjects x 3 conditions)/(2 subjects/experiment)], or more in case of subject drop-out. We have been able to re-plan and optimize the entire organization of the study to allow three subjects to be studied simultaneously. This was achieved after a) conducting a careful planning and coordination of each task during the experiments, b) devoting 50% additional research equipment to this project, c) training the experimenters to perform the various tasks (such as EEG wire-ups, etc.) in a very efficient manner, and d) running pilot studies to test the feasibility of the new approach.

While the new approach requires a remarkable planning and preparation effort and a greater coordination between all the data collection tasks, and puts greater demands on experimenters, it has the following advantages: 1) Efficiency: Reduction in the total number of experiments, hence reduced risk of loss of data and other problems; 2) Subjects studied simultaneously are each following one of 3 different sleep reduction schedules. This in turn allows to better counterbalance the possible effects of the order of presentation of the different conditions; 3) The current approach allows all subjects to complete their commitment to the project within a 5-month period, as opposed to the excessively long 7-month period in the previous approach. This in turn reduces the risk of subject drop-out; and 4) Contrary to the previous approach, subjects will always have the same partners during the three studies they will be submitted to. This greatly reduces the potential for noise in the data coming from subject interactions. We also have improved the scheduling and performance testing software program which is at the heart of the study. Certain routines have been eliminated or substituted, and others have been optimized. All the research equipment has been thoroughly tested, and new items have been acquired.

Subject recruitment and selection has been a very complex task. Subjects had to meet a series of important requirements, including: devoting 3 months of their time to the study, stretched over a 5-month period; age limitation, perfect health and fitness status; ability to endure the high demands posed upon them - dramatic sleep reduction, social confinement in a small group for a prolonged period, good educational background, etc.; and they had to be available for the study at times that were compatible not only with our labs schedule, but also with those of the other two fellow volunteers. Each of the candidate subjects was submitted to a thorough 4-night EEG and core body temperature recording period to evaluate their sleep patterns, quality of EEG, and circadian phase. They have also been asked to complete a sleep log and wear actigraphs for two weeks to monitor their pre-study sleep-wake and circadian patterns. These data enabled us to tailor the ultrashort sleep-wake strategies to the circadian phase of each individual.

The data collection conducted during Study 1 involving 3 subjects was very successful in terms of validation of the feasibility of protocols, subject compliance, and accuracy of data collected. We were particularly pleased that experiments have been completed one month ahead of the original proposed schedule. Despite the difficulty of the protocol, the sleep deprivation required upon the subjects, and the complexity of scheduling work shifts for the research staff, the study has been successfully completed without subject drop-out.

Data analyses on visual scoring of sleep recordings and analyses of the Alpha Attenuation Test are now virtually completed. Analyses of performance, subjective measures, and rectal temperature have been conducted, showing a relatively good subject compliance, and providing interesting provisional results. We are completing statistics on indices of sleep architecture. Preliminary results are described below:

A. The circadian pacemaker: Rectal temperature was recorded continuously throughout the total of 28 days in each condition. Preliminary periodograms and time series analyses revealed that the 24-hr period of the circadian pacemaker remained highly stable in all schedules (maximum q values were found at 24.00 hrs for the

polyphasic and biphasic conditions, and 24.03 hrs for the monophasic). One-way ANOVAs for repeated measures revealed no differences in the amplitudes ($p = 0.76$) nor in the acrophases ($p = 0.09$) of the temperature cycles (cosinor analyses) between conditions during the 16-day, 3-hr sleep/day intervals. These preliminary analyses suggest that under conditions of extreme sleep reduction, the circadian pacemaker maintains a very stable period of 24 hrs, without significant variations in phase and - contrary to what could be expected - amplitudes of the core body temperature cycle show no differences whether sleep is taken in one nocturnal sleep episode or divided into multiple naps during the 24 hrs.

B. Performance and subjective measures: Two-factor (sleep strategy and time-of-day) ANOVAs for repeated measures on cognitive performance tests and subjective measures have been conducted separately for each subject. A significant time of day effect has been found - as expected - for virtually all measures. We report here on the most sensitive performance measures (number correct for: Descending Subtraction, Accuracy, DSP; Grammatical Transformation, GTC; Memory and Search, MAC) and on one of the subjective sleepiness measures (Stanford Sleepiness Scale, SSS). Results for one factor are summarized in the table, which indicates in which sleep condition subjects achieved best and significant values (*n.s.* = *no significant differences between conditions*). Post-hoc pairwise Tukey's honestly significant differences tests were conducted when significance on the ANOVAs were found. Tukey's significant differences between conditions, when present, are indicated in superscript (*M=Mono*; *B=Bi*; *P=Poly*). In general, the *biphasic* condition allowed for greater efficiency, followed by *poly*.

C. Objective measure of alertness: the Alpha Attenuation Test. Our preliminary analyses focused on the calculation of AAC coefficients (ratio of eye-closed versus eye-open EEG alpha - 8-12 Hz - activity) for the P4-O2 derivation. We applied the same statistical approach used in (B), above (two-factor ANOVAs for repeated measures and Tukey's) finding a significant time of day effect. Results for one factor ANOVAs are summarized in the table, which indicates in which sleep condition subjects achieved best and significant AAC alertness levels. Interestingly, two of the three subjects achieved highest alertness levels in the polyphasic condition.

Preliminary Results Summary Table

	<u>DSP</u>	<u>GTC</u>	<u>MAC</u>	<u>SSS</u>	<u>AAT</u>
Subject 1	<i>n.s.</i>	BI ^{M,P}	POLY ^{M,B}	BI ^{M,P}	<i>n.s.</i>
Subject 2	POLY ^M	<i>n.s.</i>	BI ^{M,P}	<i>n.a.</i>	POLY ^{M,B}
Subject 3	BI ^P	BI ^M	<i>n.s.</i>	BI ^M	POLY ^M

Sleep strategies under which subjects performed best (DSP, GTC, MAC), experienced less sleepiness (SSS), or showed highest alertness (AAT) [see text for further details].

Preliminary conclusions. We have successfully confirmed that it is possible to run three subjects simultaneously and that this makes the study highly efficient. Confirming previous evidence and the main hypotheses of this project, this preliminary analyses suggests that the *monophasic* may not be the best sleep reduction strategy under sustained work. In contrast, the *biphasic* strategy with two naps per day, followed by the *polyphasic*, appear to provide the best overall effectiveness and alertness so far. It is interesting to point out that the *polyphasic* strategy provided best physiological alertness, while it also worked well in some of the performance measures. It is important to underscore, however, that the *biphasic* has the additional advantage of being a more practical approach than the *polyphasic* during certain types of real emergency situations, because the former consists of having to interrupt sustained work for sleep only twice a day as opposed to six times per day. We emphasize that these are only *very preliminary observations* and no final conclusions may be achieved before the completion of the current effort.

After completing data collection for Study 1, we started preparation for Study 2 while Study 1 data analyses were under way. Preparation included hiring and training of additional staff, subject recruitment and selection, acquisition of new equipment, re-conversion of the data collection/analyses system, and optimization of the study design.

Results from our previous and ongoing polyphasic sleep studies show that the sleep strategies proposed here may have a significant potential to overcome serious decrements of performance which may be experienced during emergencies in space flight missions. This program combines theoretical and practical interest: it will provide solutions to efficient and safe handling of emergency situations in space, while contributing to our understanding of sleep and alertness regulatory mechanisms. In addition, we will develop tools that may assist in the design of sleep-wake strategies for the growing population of individuals involved in quasi-continuous or irregular work scenarios. The specific aims of our study are: 1) To test the hypothesis that polyphasic sleep allows for dramatic levels of sleep reduction; 2) To test the hypothesis that polyphasic sleep is a practical solution to maintain high levels of efficiency under conditions of quasi-continuous work; 3) To determine the minimum amount of sleep necessary to maintain an acceptable level of performance; 4) To identify the most important factors (such as nap duration and timing, amount of prior wakefulness, nap architecture) that may affect the benefits of naps taken during extended work; 5) To further characterize the architecture of ultrashort sleep and the obligatory components of minimal sleep (e.g., slow-wave sleep, REM sleep); 6) To understand whether phase, period, and amplitude of circadian rhythms are affected by polyphasic sleep schedules. It is also expected that this study will result in significant practical application to NASA, as well as to any other organization dealing with sustained work; 7) Understanding how individuals should be trained to adapt to polyphasic sleep schedules and to develop strategies that would allow rapid transition from monophasic into polyphasic sleep. 8) Defining how individuals vary in their constitutional ability to adapt (or not adapt) to polyphasic sleep; and 9) Determining what are the limits of systematic and prolonged use of polyphasic and ultrashort sleep-wake schedules.

This research will be the first to evaluate in detail the ability of adult humans to function under an ultrashort sleep strategy. The exploration of these concepts may find its most appropriate application towards the improvement of health, safety, and well-being not only in future space missions, but also in other situations involving sustained work and/or emergency management.

It is expected that this study will form the basis for subsequent investigations to design and evaluate effective protocols for training crews for preparedness to emergencies in space (and other) missions. This project also provides an opportunity for graduate students to be trained on the fundamental skills of sleep/performance research and related applications.

FY97 Publications, Presentations, and Other Accomplishments:

Stampi, C. "Circadian rhythms, sleep, and performance in space" in "Fundamentals of Space Life Sciences." Edited by: Churchill, S.E. Krieger, FL, pp 195-211 (1996).

Stampi, C. "Plasticita' del ritmo veglia-sonno" in "Elementi di Cronopsicologia." Edited by: Cicogna, P.C. and Natale, V. Gnocchi, Napoli, pp 81-102 (1996).

Stampi, C., Moreno, C., Macchi, M., Telser, S., Sasaki, T., and O'Connor, J. "Circadian profile during ultra-short sleep schedules." Shiftwork Int. Newsletter, 14(1), 54 (1997).

Stampi, C., Von Gizycki, H., Macchi, M., Telser, S., Sasaki, T., O'Connor, J., and Leder, R. "Polyphasic vs. monophasic sleep-wake strategies under sustained operations." Shiftwork Int. Newsletter, 14(1), 40 (1997).

Cytochrome P450: Comparison of Flight Suspension

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Task Description:

Results of space flight experiments on the hepatic content of cytochrome P450, the terminal oxygenase of an important foreign compound metabolizing system in mammals, have produced conflicting observations. Since the efficacy of drugs is often dependent upon their rates of metabolism, and their safety is dependent on the rates of clearance from the body, alterations in the capacity of this enzyme system may cause decreased efficacy of drugs on one hand or increased toxicity on the other. Within the closed confines of a spacecraft, considerable potential exists for the occupants to be exposed to a variety of toxic chemicals. Alteration of these foreign compound metabolizing systems could compromise the health and safety of crewmembers.

Our previous ground-based studies indicated that the rodent suspension model does not induce significant alterations in the total activity of the P450 family of enzymes. Additional preliminary studies have included the determination of levels of a subset of individual cytochrome P450 isoforms, since these individual isoform levels could be altered without an overall change in total P450 activity. These studies utilized immunoquantitation of specific isoforms, including IIB1 and IIE1, for which specific antibodies are available. Studies completed on isoforms IIB1 and IIE1 indicated that suspension of rats significantly alters the expression of both isoforms at the protein level. The above isoforms, for which oligonucleotide probes are available, are also being assessed by Northern blotting to discern the possible mechanisms for protein alteration in suspended rats. The objectives of the current studies are to perform similar analyses on flight samples to determine the validity of the suspension model as a predictor of space flight-induced alterations in hepatic foreign compound metabolizing systems.

Liver tissue obtained from rats flown on the PARE.03 shuttle flight experiment (launch date of April 8, 1993 / recovery date of April 17, 1993) were obtained through the Biological Flight Experiments Tissue Sharing Program and included six samples from each of the following four groups: basal, ground control, flight, and flight control. Total RNA isolation was completed on liver samples and A_{260}/A_{280} ratios ($1A_{260} = 40\mu\text{g RNA}$) were obtained. RNA samples were electrophoresed in 1% agarose gels. RNA was subsequently transferred to nylon membranes and hybridized to various probes (cytochrome P450 2E1 and 2B1, 28S rRNA). cDNA and oligonucleotides used as probes were labeled and detected nonradioactively (Boehringer Mannheim). Films were

analyzed using a BioRad Molecular Analyst/Macintosh Image Analysis System. Bands for each of the three probes were quantitated in arbitrary optical density units (ODU). The ODU of bands for both cytochrome 2B1 and 2E1 films were normalized against ODU values obtained for 28S rRNA to control for potential gel loading differences. A randomized block two factor ANOVA with no interactions was used to statistically analyze the data. A Bonferoni critical value of 0.025 was used to assess significance.

Samples were in good condition upon arrival and sufficient quantities of undegraded RNA were isolated to complete the assessment of mRNA levels for each of the cytochrome P450 isoforms. A_{260}/A_{280} values in samples employed for Northern analysis were in general greater than 1.7, indicative of sufficient purity for useful measurements. Analysis of cytochrome P450 2E1 and 2B1 mRNA levels in hepatic samples from basal, ground control, flight, and flight control animals indicated there were no significant treatment effects discernible. This suggests that exposure of rats to microgravity for a period of 9 days is not associated with marked alterations in these P450 isoforms. However, this does not rule out the possibility that significant effects could have occurred at earlier times during the flight or that more prolonged flight times could be associated with such alterations. In addition, it is also possible that there were alterations in other isoforms not assessed in this investigation.

The results of this research help to characterize the influence of stress on metabolic characteristics of vertebrate systems, and have particular relevance to Earth gravity conditions with characteristics which mimic those of the microgravity environment, i.e., inactivity with its consequent musculoskeletal and cardiovascular unloading and deconditioning. In addition, the present results suggest that complex closed environments such as those on the shuttle are not necessarily characterized by pronounced effects on xenobiotic metabolism.

Visual and Vestibular Contributions to Human Heading Estimation

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Funding:

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Responsible NASA Center: Ames Research Center

Task Description:

The task of navigating through a cluttered environment involves a complex, coordinated sensorimotor process that uses visual, vestibular, proprioceptive, motor-corollary, and cognitive inputs. Determining one's movements (self-motion estimation) and the environmental layout (relative depths) are critical elements of that task. The problem becomes more acute during space flight as astronauts often work in environments where important visual cues may be missing and because microgravity induces changes in both vestibular and oculomotor function. We propose to measure and model visual and vestibular contributions to human self-motion estimation by studying heading and depth discrimination in response to pure visual (flow fields simulating self-motion), pure vestibular (actual translation in darkness), and ultimately combined visual-vestibular stimuli.

Our study of human self-motion perception is currently examining how humans process and integrate visual motion information and how eye movements relate to motion perception. Dr. Thompson at the University of York in the UK and the PI have previously identified human errors associated with low-contrast motion stimuli (such as motion obscured by fog). In FY97, we discovered that human speed perception can have significant up-down biases as well and that temporal-frequency perception has contrast-induced biases opposite those predicted from the previously identified biases in speed perception. This latter fact rules out models of human motion perception that rely on temporal-frequency perception to derive speed.

Dr. Perrone at the University of Waikato in New Zealand and the PI showed that heading judgments during simulated motion around a curve can be quite veridical even at high rotation rates. This result disproves the hypothesis put forth by others that heading estimation from visual motion is limited to rotation rates below 2°/s. The paper also resolves a series of apparent discrepancies in the field by pointing out that self-motion

estimation can be measured in either egocentric or exocentric coordinate systems and that the measurements in these different coordinates should yield different results.

Dr. Beutter of San Jose State University and the PI determined that the shape of the viewing window causes errors in the eye-movement response to motion within the window and that the errors are quantitatively related to the concurrent perceptual errors. This result provides a strong link between pursuit eye-movements and conscious motion perception, with considerable implications toward the applicability of eye-movement monitoring as an indirect but non-intrusive method of measuring the human perceptual state during aerospace applications. Furthermore, by simultaneously performing classical psychophysical measurements and measuring eye movements, we have validated a new analysis technique (oculometrics) that shows that the errors in direction perception can be quantitatively predicted from the smooth eye-movement direction.

In collaboration with Dr. Lorenceau at the College de France, Dr. Beutter and the PI showed that humans can accurately track partially occluded objects even when the correct strategy for doing so is not simply to nullify the motion on the retina. This provides a major challenge to current models of human pursuit eye movements, and we have specifically ruled out models that merely calculate the vector average of local motions. We also found additional supporting evidence for the view that perceived motion rather than physical stimulus motion drives pursuit by demonstrating a correlation between perceptual coherence and the pursuit response for stimuli perceived differently yet with the same physical motion.

Dr. Eckstein of Cedars-Sinai Medical Center, Dr. Beutter, and the PI also initiated a project to extend our examination of the link between visual perception and eye movements to include the link between static location perception and saccadic eye movements. As with the oculometrics of pursuit, we have used signal detection theory to provide a mathematically rigorous metric of oculomotor performance. Preliminary results suggest that, for detection of a target in noise, the amount of information available for saccadic targeting is the same as that available to perception.

Our study of human self-motion perception and oculomotor control has numerous significant Earth benefits. First, our model can be used to predict human performance in a variety of navigational tasks from flying to driving. Identifying situations which may lead to human error will provide information critical to engineers designing cockpits, cars, displays, and simulators, and others interested in reducing accidents (instructors, freeway designers, etc.). Second, our psychophysical paradigms will lead to better methods for measuring driver and pilot visual proficiency and for diagnosing subtle pathology in the visual system after an accident/stroke or due to aging. For example, the present method of using visual acuity to test drivers prior to license renewal does not measure the person's true ability to use visual information to navigate. The tasks we have developed to explore human self-motion perception provide a better measure of this ability. Third, our development of new technologies for measuring and analyzing oculomotor data enables the measurement of perception in real time. This new approach could be used to monitor perception in applied and real world settings where the use of standard methodologies is not possible. Fourth, because our models are based on the known physiology and anatomy of primate visual cortex, our results provide fundamental insights into how the primate brain processes and integrates sensorimotor information from multiple modalities (visual, oculomotor, vestibular) to generate a robust perception of self-motion and to guide complex motor behavior.

FY97 Publications, Presentations, and Other Accomplishments:

Beutter, B.R. and Stone, L.S. "Human motion perception and smooth eye movements show similar directional biases for elongated apertures." *Vision Res.* (In Press).

Beutter, B.R. and Stone, L.S. "Pursuit and direction perception are driven by similar and largely veridical object-motion signals." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 38, S693 (1997).

Beutter, B.R., Mulligan, J.B., and Stone, L.S. "The barberplaid illusion: Plaid motion is biased by elongated apertures." *Vision Res.*, 36, 3061-3075 (1996).

Eckstein, M.P., Beutter, B.B., and Stone, L.S. "The accuracy of saccadic and perceptual decisions in visual search." (Abstract) *Perception, Suppl.*, 26, 70 (1997).

Eckstein, M.P., Beutter, B.B., and Stone, L.S. "The visual efficiency of eye movements during search." (Abstract) *Invest. Ophthalmol. & Visual Sci.*, 38, S1152 (1997).

Stone, L.S. and Perrone, J.A. "Human heading estimation during visually-simulated curvilinear motion." *Vision Res.*, 37, 573-590 (1997).

Stone, L.S., Beutter, B.R., and Lorenceau, J. "Steady-state pursuit is driven by object motion rather than the vector average of local motions." (Abstract) *Inves. Ophthalmol. & Visual Sci.*, 38, S936 (1997).

Stone, L.S., Beutter, B.R., and Lorenceau, J. "Tracking virtual trajectories." (Abstract) *Soc. Neurosci. Abstr.*, 22, 963 (1996).

Stone, L.S., Beutter, B.R., and Lorenceau, J. On the visual input driving human smooth-pursuit eye movements. NASA Tech Brief, NASA TM 110424 (1996).

Thompson, P. and Stone, L.S. "Contrast affects flicker and speed perception differently." *Vision Res.*, 37, 1255-1260 (1997).

Thompson, P. and Stone, L.S. "Up-down asymmetries in speed perception." (Abstract) *Perception, Suppl.*, 26, 79 (1997).

Verghese, P. and Stone, L.S. "Spatial layout affects speed discrimination." *Vision Res.*, 37, 397-406 (1997).

Development of an Advanced Video Ocular Measurement System

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

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Responsible NASA Center: Johnson Space Center

Task Description:

Video-based tracking systems have come into prominence in several research fields as the preferred eye measurement tool because of the precise multidimensional analysis capabilities they provide. The goal of this project is to build a real time Video Ocular Measurement System (VOMS) based on the prototype VOMS which implemented a new video eye image analysis algorithm for the STS-42 Microgravity Vestibular Investigations (MVI).

The new video eye image analysis algorithm, called the disk-fitting algorithm, was developed under the joint efforts of NASA and the University of Michigan. It tracks four dimensional ocular parameters, including the horizontal and vertical coordinates of pupil centers, pupil sizes, and torsion angles. The resolution of the measurements is less than 0.05 degrees for the pupil center tracking, and about 0.3 degrees for the torsion angles. Despite its capability to provide these extended measurements, the prototype VOMS is handicapped by its low computing power, geometric distortion, and poor user interface. Therefore, the prototype system has been operated in an off-line processing mode.

We propose to implement the new disk-fitting algorithm on a Power-PC based personal computer since a test proved that such PCs have adequate power for the real time execution of the disk-fitting algorithm. New image handling, geometric correction, and user-interface routines will be incorporated for a complete system.

The software modules for analyzing the eye images were assembled and tested. During the course of the system implementation, it was discovered that the multi-processing application programming interface for the Power-PC based computer platform carried significant amount of processor overhead, and didn't perform as anticipated. The assembled software is running pseudo-real time mode on the computer platform which was selected at the starting phase of this project. Currently, much faster platforms are available. The new interface and the programming structure of the software will be ready to use for those faster platforms with minor adjustment.

The purpose of this project is to build a system that can accurately measure human eyeball movement. Since eye movement data carries important neurological information under certain circumstances through vestibulo-ocular reflex, an apparatus providing accurate and noninvasive measurement, such as the proposed system, will become an invaluable tool for neuroscientists, no matter whether in space or on Earth.

Other than the originally proposed application area, the eye movement data can be used in many different capacities. Clinical medicine, hand-free computer/machine interface, and target aiming aid could benefit from automatic eye movement tracking. The system technology developed in this project will serve as a seed technology for expanding eye movement tracking into other application areas. The possibility of the spin-off of the proposed system to the commercial sector would be enormous.

FY97 Publications, Presentations, and Other Accomplishments:

Sung, K.J. and Reschke, M.F. A model-based approach for the measurement of eye movements using image processing. NASA Tech Brief 1-39 (1997).

Digital Echocardiography in Manned Space Flight: Remote Diagnosis and Quantitative Analysis

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Funding:

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Students Funded Under Research: 2
FY 1997 Funding: not available

Solicitation: not available
Expiration: 1999
Post-Doctoral Associates: 3

Task Description:

Accurate assessment of the cardiovascular system during manned space flight is important for two broad reasons. First, it is essential that diagnostic information be available for rapid assessment of cardiac function during a medical emergency. The need for such assessment will grow exponentially as humans venture farther from the Earth, with flight times anticipated to be greater than one year for travel to Mars. Second, our understanding of the cardiovascular adaptation to microgravity remains imperfect. While prior studies have demonstrated relatively minor changes in cardiac volumes and output, the development of orthostatic hypotension and cardiovascular deconditioning remains problematic. An improved methodology for assessing subtle changes in the central and peripheral cardiovascular system under microgravity may, therefore, aid in developing improved strategies for adapting to the microgravity environment. In order to address the broad goals of this research and to overcome the above two limitations, we will pursue four specific aims: (1) Develop the infrastructure for digital acquisition, storage, and transmission – both terrestrial and by satellite – of echocardiograms; (2) Test existing and novel digital compression algorithms for their impact on clinical interpretation and quantitative content; (3) Validate new quantitative approaches to assessing cardiovascular mechanics in microgravity: (A) Color Doppler M-mode echocardiography, and (B) Doppler Tissue Imaging; and (4) Refine the acquisition, segmentation, display and analysis of three-dimensional echocardiograms.

The earliest space-based application of these aims will be towards perfecting the methodology for digital transmission of ultrasound images from the International Space Station (ISS) to clinical and research specialists at the Cleveland Clinic Foundation and elsewhere. Through the knowledge gained from Specific Aim #3, we will propose novel observational experiments to be conducted in the Human Research Facility of the Space Station to investigate the adaptation of the cardiovascular and musculoskeletal system to prolonged microgravity. This work will also be immediately applicable to terrestrial medicine, standardizing the technology for digital transmission of echocardiographic data both within a hospital and remotely for telemedicine. Extending the temporal horizon to 5-8 years, the work of Specific Aim #4 (by its use of three-dimensional acquisition which requires less operator expertise than 2D echo) will impact significantly further ultrasound investigation on the Space Station and improve the delivery of medical diagnosis on longer

missions to the moon, Mars, and beyond. It is therefore hypothesized that this proposal will be of unique value to NASA in its delivery of space-based medical diagnosis and investigation of microgravity physiology while delivering tangible benefit to American and world society by improving the value of echocardiographic services in cardiovascular care.

Specific Aim #1

- Ordered Nova Microsonics upgrade of existing components for digital echocardiography
- ATL research link enabled on HDI3000 to increase ease of digital network transfers
- HDI-Lab software installed on PC to allow network transfer of echocardiographic image sequences
- Working towards an agreement to participate in ATL's clinical trial of automated boundary detection (ABD) software and installation of SGI ABD software for clinical trail
- Training of research sonographers to work with ATL research link, PC and SGI software
- Evaluation of HDI5000 and preparation of science evaluation regarding this upgrade to planned space station installation
- Presentation of ultrasound upgrade proposed to HRF Science Working Group (7/28/97)
- Presentation of ultrasound upgrade proposed to Ultrasound CDR (8/11/97)
- Coordination with Lockheed-Martin engineers to develop digital file transfer from ISS

Specific Aim #2

- Planning with Dr. Kim Boyer (Electrical Engineering, Ohio State University) to begin subcontract. A graduate student (still to be named) will work with Kim on echocardiographic image compression. An undergraduate student will also be funded in this area. Transfer of echocardiographic datasets to OSU for processing and analysis.
- NREN trial in collaboration with NASA Lewis and Ames performed
- 8/28 Live Ultrasound transmitted at 2-5 Mbps via ATM using MPEG compression with varying cell loss and cell error ratios
- 9/14 Demonstration at NREN Conference (Ames) of live echo transmission from Lewis

Specific Aim #3

- Visit to Dr. David Kass' Lab at Johns Hopkins, Baltimore for evaluation of conductance catheter system and discussion of calibration and use issues
- Human OR Hemodynamic - Echocardiographic studies (ten cases)
- Evaluation of prototype *In vitro* Compliant model of LV filling, plan further refinement of model
- Meeting in Gent, Belgium with Dr. Pieter Vandervoort (consultant)
- Presentations at the Computers in Cardiology Conference, Lund, Sweden
- OSU Graduate Student (Xiyi Hang) to begin at CCF in November

Specific Aim #4

- Project Staff position filled - Dr. Takahiro Shiota
- Candidate identified and interviewed for post doctoral position in 3D image processing – offer to be made in conjunction with BME position after completing their interview process with his wife
- 3D ultrasound machine has been purchased and delivered in September--Clinical value demonstrated in case of obstructed left ventricular assist device
- Partnership established with Duke - North Carolina National Science Foundation / Engineering Research Center for Emerging Cardiovascular Technologies
- 3D software development:
LabVIEW 3D ultrasound file reader implemented
Ross Lab (Kevin Montgomery) C++ 3D ultrasound reader, 3D visualization
Onyx system (CCF - Neurosurgery) to be used to test/evaluate Duke software
- Evaluation of SGI 02 and Octane Workstations

Although the principal impetus for this proposal is improving space-based diagnoses, each of these specific aims has important practical and economic implications for the health care in the United States and the rest of the world. Echocardiography, an exceedingly versatile test for the assessment of cardiovascular morphology and

hemodynamics, is the most common cardiac imaging test, and constitutes the single largest component of the Medicare budget in cardiology. In 1994 (the last year for which complete data are available), Medicare expenditures for echocardiographic services exceeded \$1,040,000,000. Extrapolating this to all health care payers, we estimate that over 5,000,000 echocardiograms are performed annually in the United States at a cost approaching \$3,000,000,000. Clearly, any strategy that a) decreases the needless repetition of tests, b) increases the efficiency for performing and interpreting a given test, or c) improves the quantitative value of a single test has the potential for tremendous savings to the American health care system.

In this proposal, we have sought to develop new methods to optimally deliver echocardiographic services by way of digital storage and transmission of images and quantitative three-dimensional acquisition, display, and analysis. The key benefits to terrestrial health care will be: a) clinical validation of the acceptability of digital compression of echocardiograms; b) research and development of novel compression schemes; c) software development to allow echocardiographic exchange over land- and satellite-based networks and the World Wide Web; d) application of these concepts to real-time three dimensionally acquired echocardiographic data; and e) propagation of this work to the echocardiographic, general medical, and biomedical engineering communities.

If these initiatives are successful, we estimate that at least 5% of currently performed echocardiograms will be rendered unnecessary (saving \$150,000,000); digital retrieval should speed physician review, allowing the cost (in physician time) to be reduced by 10% (reducing total resource use per echo by about 5%, achieving \$135,000,000 in additional savings); and finally, extension of these approaches to three-dimensional echocardiography will greatly enhance the value of these tests, allowing them to replace more expensive nuclear cardiology and magnetic resonance imaging tests.

FY97 Publications, Presentations, and Other Accomplishments:

Bajzer, C.T., Greenberg, N.L., Arheart, K.L., Vandervoort, P.M., and Thomas, J.D. "Evidence of regional differences in left ventricular isovolumic rate of relaxation in a canine model." (Abstract) *J. Am. Coll. Cardiol.*, 29(2), 214A (1997).

Ballal, R.S., Secknus, M.A., Mehta, R., Kapadia, S., Lauer, M.S., and Marwick, T.H. "Cardiac outcomes in coronary patients with submaximal dobutamine stress echocardiography." *Am. J. Cardiol.* (In Press).

Brar, R., Yurow, G., Neumann, A., Stamos, T., Garcia, M., Stoddard, M., Cherian, P.K., Thomas, J.D., and Soble, J.S. "Comparison of MPEG digital image compression with super VHS for diagnostic echocardiographic readings." (Abstract) *J. Am. Soc. Echocardiogr.*, 10, 409 (1997).

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Inflammatory and Mechanical Components of Muscle Injury

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Post-Doctoral Associates: 6

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Task Description:

The ability of personnel to function following space flight is limited by debilitating muscle weakness, pain, and inflammation that develop following return to gravitational loading. In this investigation, we are using the rat hindlimb suspension model to determine whether these injuries are attributable both to mechanical effects and to muscle damage by inflammatory cells. Our preliminary findings support the following, hypothetical injury mechanism. Muscle loading following periods of unloading causes mechanical damage to a small population of muscle fibers resulting in release of factors from injured cells that attract inflammatory cells. Neutrophils, which appear at higher concentrations in muscle at 2 hours following the onset of reloading, are the first inflammatory cells to arrive in the muscle. Neutrophils then induce further damage to reloaded muscle fibers by complement-mediated membrane damage and by reactive oxygen intermediates (ROIs). Most complement and ROI-induced damage occurs between 2 and 12 hours following initiation of reloading. At 12 hours reloading, phagocytic macrophages appear at elevated concentrations in muscle and invade injured fibers. At 24 hours reloading, macrophages associated with muscle repair appear at high concentrations.

We are testing this hypothetical mechanism by manipulating loading conditions following hindlimb suspension so as to determine whether muscle damage during the peak injury period is attributable to mechanical loading or to inflammatory cell actions. We will also test the hypothesis by performing suspension/reloading on neutropenic rats to determine whether absence of neutrophils influences the severity of muscle damage. The role of specific, neutrophil-derived mediators of cell damage will also be assayed. Finally, we will use an *in vitro* model of muscle loading to test whether loading muscle in the presence of inflammatory cells results in more muscle damage than when muscle cells are loaded in their absence.

The results of this study can be of substantial importance to the mission of NASA by determining whether there is a central role for inflammatory cells in causing muscle injuries following space flight. Substantiation of this role for inflammatory cells will indicate that pharmaceutical approaches to control their activation during muscle reloading can ameliorate many of the debilitating effects of space flight on muscle.

Our previous findings showed that inflammatory cells are responsible for much of the muscle fiber injury that occurs when muscle is reloaded following periods of reduced loading. We are currently evaluating the role of the complement system in muscle injury following modified loading by evaluating muscle injury in rats that have

received intravenous administration of recombinant soluble complement receptor 1 (sCR1) that blocks activation of the alternative and classical complement cascades. We are also evaluating the contribution of reactive oxygen intermediates to muscle injury during modified loading in rats that have received intravenous administration of superoxide dismutase and catalase (SOD/CAT) to rats during hindlimb suspension and reloading to test whether superoxide contributes to muscle fiber injury during reloading. The results of these *in vivo* studies are being extended to *in vitro* analyses in which we are examining the cytotoxicity of select populations of inflammatory cells, especially neutrophils, on muscle cells in co-culture. Through these *in vitro* analyses utilizing pharmacologic agents that inhibit the production or scavenge specific populations of free radicals that are generated by inflammatory cells, we are characterizing the free radical based mechanisms through which inflammatory cells can kill muscle cells. These *in vitro* investigations will be followed by studies in which we test whether mechanical loading regulates the production of free radicals by inflammatory cells that are implicated in muscle lysis.

Our investigation, in which we are examining the contribution of inflammatory and mechanical factors to muscle injury following modified muscle use, is expected to be broadly relevant to understanding muscle injury and inflammation. If our findings substantiate our hypothesis that inflammatory cells are responsible for most muscle fiber injury that occurs during modified muscle loading, this will indicate that these muscle injuries can be controlled by the prophylactic use of anti-inflammatory drugs. This finding would be of practical value not only to returning astronauts, but also to individuals experiencing changes in musculoskeletal loading at 1-G. For example, this new knowledge would be of value in designing therapeutic approaches to control muscle injury during reambulation of patients following prolonged bedrest.

FY97 Publications, Presentations, and Other Accomplishments:

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Tidball, J.G. (invited discussant) "Pharmacological approaches to Duchenne muscular dystrophy." Duchenne Muscular Dystrophy Conference. University of Pittsburgh, PA (1997).

Tidball, J.G. (symposium speaker) "Mechanical design in organisms: Transduction of mechanical information to cells." Society for Integrative and Comparative Biology, Albuquerque, NM (1997).

Tidball, J.G. (symposium speaker) "Structure and regulation of cytoskeletal-membrane interactions in skeletal muscle." Federation of American Societies for Experimental Biology, New Orleans, LA (1997).

Adaptive Plasticity of Otolith-Ocular Responses

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Responsible NASA Center: Ames Research Center

Task Description:

Otolith signals of gravity and translational acceleration during head motion travel on vestibular afferents to central vestibular pathways to control vestibulo-ocular reflexes (VORs). VORs maintain eye position in space and stable vision during motion by generating compensatory eye motion. VORs are not isolated motor reflexes, but function with visual and somatic mechanisms that control head orientation on the body, and posture and locomotion. VORs are part of a sensorimotor orientation system permitting accurate, effective function in 3-D space. Normally, this system enables goal-directed motion, identification/following of visual targets, and identification/manipulation of external physical objects. Change in any part of the system (e.g., otolith function change in microgravity) impacts our ability to orient in 3-D. Understanding the nature of LVORs and how they adapt to environmental change motivates the proposed studies, and defines their relevance to NASA.

LVORs occur during motion along interaural (IA), naso-occipital (NO), and dorso-ventral (DV) head axes. LVORs include: 1) Two during motion along axes perpendicular to the line of sight, horizontal responses to IA-axis and vertical responses to DV-axis motion (both compensate for head motion); 2) Compensatory vertical and horizontal eye motion occurs during NO-axis motion (along axis parallel to the line of sight). Such LVORs are small or absent when gaze is straight ahead, and increase as gaze becomes more eccentric. Response phase reverses for gaze to right versus left, or up versus down; 3) For compensatory LVORs, target distance (vergence) is a potent influence; near targets require larger eye motion than more distant ones for similar head motion; and 4) Compensatory LVORs combine instantly to produce eye motion compensatory for motion along axes between IA, NO, and DV, indicating that LVOR neural circuits integrate information about vergence, eye position, and otolith outputs.

LVOR plasticity has been demonstrated following space flight and after exposure to altered visual inputs. Following an 11-day flight, 2 rhesus monkeys showed changes in the relationship between vergence angle and sensitivity to IA or DV motion, and deficits in maintenance of behaviorally appropriate eye motion during head

movement along axes between IA and DV. Following a 60 minute exposure to left/right displacing prisms, NO-axis LVOR kinematics was altered appropriately for gaze.

Results of experiments on neural mechanisms underlying LVORs have been published. These studies were designed to examine the role of irregular and regular afferents in modifying LVOR characteristics during linear and angular stimulation. They used electrical stimulation through labyrinthine stimulating electrodes to selectively and reversibly functionally remove irregularly discharging afferent responses. This procedure makes afferents unresponsive to head movements for as long as the currents are present. The experiments provided background data showing that this tool has potential use in studying the underlying neural mechanisms of the otolith-ocular reflexes (Minor *et al.*, 1997). Additional experiments were performed to enhance our understanding of the effect of environmental enrichment on the well-being of the animal subjects of these experiments. These experiments were motivated by our continuing interest and care about animal welfare. Some results of those experiments have been published (Spring *et al.*, 1997), and some are in preparation for publication. The basic finding was that environmental enrichment devices added little to animal well-being, but group-housing, which provides increased opportunities for social interaction, significantly reduced signs of animal stress. We continued to analyze results in preparation for publication in a third area of research related to our vestibulo-ocular work. That is in further analysis of data on the potential effects of animal physical restraint identical to that experienced in space flight, and the use of behavioral enrichment devices, on heart rate and variability, a sensitive indicator of animal "comfort." Our results have shown that heart rate increases over a 20 day period without plateauing, and that the behavioral enrichment device did not provide improvement (Clifford *et al.*, 1996).

This task has for the past 6 years addressed mechanisms of oculomotor control by the little-understood, gravity-sensing otolith organs of the body's equilibrium system. Research has required the use of the unique centrifuges and linear sleds of the Ames Vestibular Research Facility. Results of our studies have encouraged clinicians to study the otolith-ocular reflexes in human clinical populations, and have demonstrated that increasingly sophisticated clinical testing of specific otolith function can be done in humans. The research sponsored by this task led to the first demonstration of specific otolith-ocular reflexes in response to linear head motion, and the first demonstration that those reflexes are plastic, adapting to different stimulus challenges in much the same way as angular vestibulo-ocular reflexes do. Aging and space flight both require sensori-motor changes that stimulate neurological mechanisms to restore, or to compensate for, compromised function. In the elderly, natural aging involves slow structural deterioration, but the consequent loss of function may be considerably hastened by acute disease, such as stroke. In astronauts, contextual changes occur soon after liftoff and 'de-conditioning' accompanies prolonged exposure to microgravity. As in the aged, such deconditioning is marked by homeostatic changes that compensate for reductions in normal function. The research in this task has been motivated by the hope that better understanding of otolith mechanisms will enable human beings, whether astronauts or just plain folks, to get better medical treatment, and the desire to contribute important new information to the basic corpus of biomedical knowledge.

FY97 Publications, Presentations, and Other Accomplishments:

Clifford, J.O., Tomko, D.L., Sueoka, S.H., and Welch, R. "Cognitive enrichment's ability to reduce psychological stress during restraint." *Psychonomic Soc. Abstr.*, 1, 33 (1996).

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Pharmacological Intervention to Prevent Disuse Osteopenia

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Post-Doctoral Associates: 1

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Task Description:

We propose that disuse osteopenia results in large measure from disturbed expression of skeletal signaling molecules (e.g., TGF- β). These signaling molecules are not exclusively regulated by weight bearing; they serve as intermediates in the signal transduction pathways for many physiological regulators of bone metabolism (e.g., calcium regulating hormones). As a result, it should be possible to bypass the mechanoreceptor step in the signal transduction pathway induced by dynamic weight bearing. This goal could be accomplished with pharmacological agents which modulate expression of the same skeletal signaling molecules (e.g., growth factors) as physical activity. Specifically, we propose that pulsatile administration of parathyroid hormone (PTH) will prevent disuse osteopenia. Furthermore, we predict that dynamic weight bearing and PTH each act to stimulate bone formation by regulating the expression of transforming growth factor- β (TGF- β) in skeletal tissues.

The goals of the two year proposal are to 1) establish a method to administer PTH during space flight which does not require astronaut intervention, and 2) determine if the anabolic action of PTH on bone formation requires weight bearing. The first goal will be accomplished by implantation of Alzet osmotic pumps that are loaded to deliver pulsatile release of PTH. The second goal will be realized by testing the effects of PTH on bone formation in hindlimb unloaded rats. In the latter study, the effects of the hormone on unloaded hindlimbs will be compared to the effects on the loaded forelimbs.

The first specific aim was accomplished and the results are now published (*Endocrinology* 188:4607-4612, 1997). We have performed the rat studies to determine the efficacy of PTH in preventing cancellous osteopenia in an unloaded limb (Specific Aim 2). We are in the process of measuring the bones. If positive results are obtained, a compelling argument could be made to further investigate the potential for using intermittent PTH therapy to reduce space flight-induced bone loss.

Although these studies were directed at developing a method to administer PTH to laboratory animals during space flight, the results are relevant to human disease. PTH treatment holds great promise as a therapy to treat several common forms of bone loss including postmenopausal osteoporosis. Potential problems of PTH therapy include cost, detrimental side effects, and the requirement to endure frequent injections. The important side effects of PTH treatment consist of hypercalcemia and skeletal abnormalities.

It is known that continuous exposure to PTH is detrimental, but the precise therapeutic "window" of exposure is not known. Our results are important because they demonstrate that PTH cannot remain in circulation for intervals much in excess of 1 hour without serious consequences. Furthermore, we have shown that it is possible in theory to administer PTH discontinuously using a simple implantable device. As a consequence, it may be possible to evoke the full therapeutic actions of PTH without the need for frequent injections.

FY97 Publications, Presentations, and Other Accomplishments:

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Reconstructions and Representations of Cerebral Cortex [Human Brain Project]

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Funding:

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Students Funded Under Research: 3	Post-Doctoral Associates: 1
FY 1997 Funding: not available	
Joint Agency Participation: NIH and Human Brain Project	

Task Description:

Neuroscientists have obtained vast amounts of experimental data about the organization and function of the cerebral cortex in non-human primates, especially the macaque monkey. To cope with this flood of information, new tools and strategies are necessary in order to adequately analyze and communicate these findings. To this end, we propose a collaborative effort that brings together scientists with complementary expertise in neuroanatomy and image processing and capitalizes on access to high-performance parallel computing resources and high-speed networking capacities. Our common goal is to develop and apply a family of interrelated computer graphics programs to be used for representing information about cortical structure and organization. The integrated system will allow visualization of three-dimensional (3-D) reconstructions of the entire cerebral hemisphere that are based either on volumetric representations or on selected surface contours. These reconstructions will be used to display information about the location of different cortical areas as well as data from specific experimental procedures. To compensate for the marked differences between individual brains, we will develop warping algorithms that can accurately transform one brain into the shape of another. These transformations will be based on probabilistic approaches to shape modeling that have had considerable success in other domains of biology. We will also develop computerized techniques for making unfolded representations of the cortex. These techniques will be used to generate comprehensive, easily updatable summaries of different schemes for the layout of various areas throughout the cerebral cortex. These will in turn be used as the framework for a graphically oriented database of the connectivity of different areas. Collectively, these approaches will greatly enhance the accuracy, speed, and flexibility with which many types of information about cortical organization can be represented and communicated. In addition, it will provide a much needed framework for more accurate comparisons with the human brain.

Our collaborative effort to develop and apply new approaches to the mapping of primate cerebral cortex has progressed on multiple fronts. A common theme underlying these efforts is the use of explicit surface reconstructions to represent the shape of the highly convoluted cortical sheet. In particular, we have used computerized surface reconstructions for surface-based visualization, surface-based warping, and the establishment of surface-based atlases of human and monkey cortex. The surface-based atlas of the Visible Man is intended for widespread use as a framework for displaying the location of distinct cortical areas and regions of functional specialization obtained from neuroimaging studies in many different laboratories. To assist in this purpose, we developed a method for mapping data from standard stereotaxic (Talairach) space onto the Visible Man atlas that includes an explicit representation of the associated spatial uncertainty. In addition, we have used surface-based warping techniques for more accurate mapping onto the Visible Man atlas in experiments where fMRI data are accompanied by cortical surface reconstructions. An analogous surface-based atlas of the macaque monkey will serve as a template for charting the layout of cortical areas revealed by numerous anatomical and physiological studies. We have also applied our surface-based warping algorithm to deform a flat map of the macaque to match the shape of the human flat map, thereby facilitating the evaluation of potential homologies in cortical areas in the two species. The two atlases and the associated visualization software (CARET and its web-based counterpart, CARETdaemon) have recently been made freely available and are in use in a number of other laboratories. Finally, we have continued the development of a prototype database that will be used to allow ready tracking and access to the large numbers of surface reconstructions, volume reconstructions, and associated experimental data that have been obtained in ours and other laboratories.

Our research objective is to generate an integrated family of brain-mapping tools for studying the organization and function of the cerebral cortex in primates. The cerebral cortex is the dominant structure of the human brain and is largely responsible for our uniquely human capabilities for perception, language, and higher cognitive function. Vast amounts of information are becoming available about the human cerebral cortex, particularly with the advent of powerful new functional brain imaging approaches. This includes extensive information about cortical organization and function in states of disease or mental disorder, as well as for normal, healthy humans. Complementing these human studies is an explosion of information about the cerebral cortex in non-human primates, which can be studied intensively with a variety of anatomical, physiological, and behavioral techniques. In order to analyze, interpret, and communicate this flood of information properly and effectively, new techniques in the area of computerized brain mapping are critically needed. Our methods for computerized reconstructions and flattening the cerebral cortex represent important tools that are being made freely available to the neuroscience community. They will allow the brain to be studied at higher spatial resolution and with better means of visualization than was previously possible. Our strategy of using shape-based deformation algorithms represents a powerful alternative to conventional methods for compensating for the high degree of individual variability in the size, shape, and pattern of convolutions of the cerebral cortex. The graphically oriented database we are developing, once it is ready for distribution, will greatly improve access of the international neuroscience community to critical, up-to-date information about cortical organization and function in humans and laboratory animals. Altogether, we envision that the contributions of this project will substantially accelerate our ability to understand the human brain in health and disease. This progress will also enhance our ability to study how an enclosed zero-gravity environment can affect human brain function and to develop strategies to minimize or compensate for the deleterious effects of living and working in space.

FY97 Publications, Presentations, and Other Accomplishments:

Drury, H.A. and Van Essen, D.C. "Analysis of functional specialization in human cerebral cortex using the visible man surface based atlas." *Human Brain Mapping*, (special issue) 5, 233-237 (1997).

Drury, H.A., Van Essen, D.C., Snyder, A.Z., Shulman, G.L., Akbudak, E., Ollinger, J.M., Conturo, T.E., Raichle, M., and Corbetta, M. "Warping fMRI activation patterns onto the visible man atlas using fluid deformations of cortical flat maps." *NeuroImage*, 5, S421 (1997).

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High-resolution Digital Mammography/NCI

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Initial Funding Date: 1995

Expiration: 1997

Students Funded Under Research: 3

Post-Doctoral Associates: 2

FY 1997 Funding: \$250,000

Joint Agency Participation: NIH

Task Description:

The specific aims of this project are focused on the development, optimization, and pre-clinical evaluation of a scanning slot x-ray detector for digital mammography. The technical objective is to achieve the following imaging characteristics of the scanning slot x-ray detector: (1) >80% quantum absorption efficiency; (2) 15 lp/mm limiting spatial resolution; (3) ~0.5% contrast sensitivity; (4) >70% zero spatial frequency detective quantum efficiency (DQE(0)); (5) >400:1 dynamic range; and (6) imaging a 20 cm x 24 cm breast in about four seconds. The primary goal is to develop a CsI:Tl screen-based scanning slot x-ray detector for digital mammography. The detector will be made of eight CsI:Tl screen-image guide-CCD modules, which form a total detector cross sectional area of 0.8 cm x 20 cm. The CsI:Tl screen is made of prismatic type CsI crystals of ~5 microns in diameter. This fiber-like structure serves to limit the spread of the scintillation light which permits the use of a relatively thick CsI:Tl screen to improve detector x-ray interaction efficiency without sacrificing resolution performance. In addition, the scintillation decay time of CsI:Tl is ~1 microsecond which eliminates the afterglow effect associated with the use of a rare-earth phosphor screen in a scanning slot detector. The image guide will be made of clear plastic optical fibers of ~6 microns in diameter. The input area to output area ratio of the image guide will be 1:1. Each CCD, operated in the time-delayed integration (TDI) mode, is an 1100 x 330 pixel array with pixel size of 24 microns. Pixel charges from all the CCDs are read out in parallel and digitized to 14 bit. A real-time flat-fielding unit will be implemented to correct detector non-uniformity. The final image format is 8K x 8K.

The performances of the CsI:Tl screen based scanning slot x-ray detectors will be quantified by measuring the detector contrast sensitivity, modulation transfer function (MTF), noise power spectrum (NPS), and DQE. Phantom tests will be performed to measure the detector non-uniformity and artifacts. A comparative receiver operating characteristics (ROC) analysis will be performed to evaluate the performance of the CsI:Tl screen-based scanning slot x-ray detector and a conventional screen-film mammography system. Simulated features (masses and microcalcifications) will be randomly positioned on an anthropomorphic breast phantom to produce different image backgrounds around the simulated features. Radiologists' performance to detect the presence of masses and microcalcifications will be evaluated to compare the digital system and the screen-film system.

Our preliminary tests of a CsI:Tl screen based slot detector have shown a limiting spatial resolution of ~15 lp/mm. By the use of plastic fiber image guides, it is possible to construct a high quality digital mammography system which will be very competitive with the other technologies using expensive glass fiber image guides.

- 1) A circuitry for real-time correction of CCD dark current and detector non-uniformity has been developed.
- 2) The spatial resolution and signal transfer properties of a CsI:Tl screen-based slot detector have been measured. The results demonstrated that high spatial resolution and high detective quantum efficiency can be obtained by the use of a CsI:Tl screen as the x-ray-to-light converter in a slot x-ray detector for mammography.
- 3) Plastic optical fiber image guides are being developed for use in the CsI:Tl screen-based slot detector. These image guides will be made of ~6 micron diameter plastic fibers with 1:1 input area to output area ratio. The use of ~6 micron diameter fibers in the image guides is essential to maintain high detector spatial resolution. Compared to the use of expensive glass fiber image guides, the use of these plastic fiber image guides will significantly reduce the cost of a scanning slot digital mammography system. This work is benefited from a research project funded by a DRAPA grant. In that project, Nanoptics, Inc. is developing an ultra high purity optical polymer production process. The monomer is filtered sequentially down to 0.01 microns then processed in a high level plate distillation system to ensure extreme purity. The ultra pure monomer is then polymerized and extruded into fiber in a closed system reactor and extrusion facility. The digital mammography project will use this DRAPA facility to produce plastic fibers with diameter down to 2.5 microns and high transmission and no blemishes.

This research is to develop a digital x-ray camera system which can be used to perform radiological screening for detection of very early breast cancer. At present, about 25% of all breast cancer is missed in screening women using the existing mammographic systems. Due to the very high spatial resolution and contrast sensitivity, the camera will be significantly more sensitive to the earliest signs of the disease.

This technology for digital radiology can be easily extended to general radiology for the chest and major organs. In this case, the typical x-ray energies are increased from about 20 keV to 80 keV. The real-time nature of image acquisition and display is particularly important for trauma or battlefield patients.

There are major applications of large area, high resolution, real time digital radiographic cameras for industrial, aeronautical, and space applications. High performance, composite materials are increasingly being used in these industries. This technology can meet the required specifications to optimize the processing and perform quality control of components made of these new materials.

There are also a number of applications where the high resolution high transmission plastic fiber image guides can be used: (1) High resolution image guides/tapes/fiber optical plates for medical and industrial applications; (2) Space application from the benefit of low weight image guides; and (3) Heads-up virtual reality image guides where low weight high resolution is important.

FY97 Publications, Presentations, and Other Accomplishments:

Jing, Z. Imaging performance of scanning slot x-ray detectors for digital mammography. (Dissertation) University of Florida (May, 1997).

Jing, Z., Huda, W., and Walker, J.K. "Scattered radiation in scanning slot mammography." *Med. Physics* (In Press).

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Investigation Of Laser-Polarized Xenon Magnetic Resonance

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Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 2

Post-Doctoral Associates: 2

FY 1997 Funding: \$ 118,196

Task Description:

We propose ground-based investigations of a new biomedical diagnostic technique: the inhalation and magnetic resonance (MR) of laser-polarized spin 1/2 noble gases: ^{129}Xe (xenon) and ^3He (helium). Laser-polarized noble gas MR may allow the imaging and spectroscopy of human body structures and functions that have heretofore been poorly resolved by conventional proton MR, either because of low signal level and structural artifacts (e.g., the lung and lipid membranes), low signal contrast (e.g., differential perfusion studies), or poor tissue specificity (e.g., chemical shift MR). Laser-polarized noble gas MR is a recently demonstrated technology that may have important biomedical applications, including: (i) detailed lung imaging; (ii) the measurement of blood flow to tissue (perfusion); (iii) the characterization of lipid membrane integrity (e.g., in multiple sclerosis); (iv) chemical shift imaging and spectroscopy; and (v) the study of anesthesia. In addition, noble gas laser-polarization occurs external to the body, prior to the inhalation of the gas. As a result, at very low magnetic fields (~ 0.001 tesla) the signal-to-noise ratio (SNR) of laser-polarized noble gas MR can be much larger than one, whereas the SNR of proton MR is less than one. The creation of such very low magnetic fields is practical in a small, low-power device. Therefore, since the production of laser-polarized gas should be possible in a small, low-cost package, very-low-field noble gas MR may enable both portable ground-based and practical space-based biomedical MR systems.

Development of a large-scale polarization system employing diode laser arrays

We developed a diode-laser-array polarization system to produce approximately one liter per hour of hyperpolarized ^3He or ^{129}Xe gas. This large-scale polarization system incorporates four 20 watt diode laser arrays. Each laser is fiber-coupled, and then the four fiber bundles are merged into a single meta-fiber bundle that provides up to 80 watts of optical pumping light onto the polarization chamber. A compact optics package has been developed that allows > 90% of the power from the diode laser array system to be circularly polarized and projected through the volume of a polarization chamber ~ 0.5 m in length. The polarization system (including pumps, lasers, etc.) is mounted on a standard laboratory cart and can be easily moved in and out of an MRI facility as needed. Performance and reliability tests of this system are underway.

Investigation of ^{129}Xe polarization with diode laser arrays

We performed model calculations of the ^{129}Xe hyperpolarization process. We are investigating how ^{129}Xe polarization depends on gas pressures and mixtures, cell properties, temperature, optical pumping light, etc. In parallel, we are developing a ^{129}Xe polarization test-bed to study the effect of the aforementioned system parameters and compare with model calculations. The results of these studies will be used to improve the large-scale noble gas polarization system described above.

Investigation of *in vitro* tissues

In collaboration with researchers at the Brigham and Women's Hospital, we measured the polarization lifetime (T_1) of laser-polarized ^{129}Xe dissolved in fresh human blood *in vitro*. A blood-foam preparation was used to enhance the MR signal of ^{129}Xe dissolved in blood. We found that the dissolved ^{129}Xe T_1 is significantly shorter in oxygenated blood foam than in deoxygenated blood foam. To understand the oxygenation trend, T_1 measurements were also made on plasma and hemoglobin foam preparations.

Development of polarized noble gas MR imaging techniques

In collaboration with researchers at the Brigham and Women's Hospital, we investigated gradient echo imaging strategies for laser-polarized ^{129}Xe MRI. We performed experiments on the use of different gradient echo pulse sequences and found that a variable flip angle approach can improve the ^{129}Xe signal to noise ratio and eliminate some typical image artifacts. We also demonstrated that although a constant signal intensity can be obtained with such an approach, the maximum spatial resolution achievable is constrained by the ^{129}Xe polarization lifetime, T_1 .

Low field noble gas imaging

We developed a low magnetic field system for laser-polarized noble gas MR. For laser-polarized noble gases, the ensemble magnetization does not depend on the applied magnetic field — unlike conventional, thermally polarized systems. Thus at low magnetic fields (< 100 gauss), water proton MR signals are not observable without extensive signal averaging; whereas laser-polarized noble gas MR images can be obtained clearly and rapidly in a single measurement. Recently, we made the first low-field MR images of laser-polarized noble gas: for example, we have obtained images of laser-polarized ^3He contained in glass cells at 20 gauss. These images have been acquired using a simple, low-flip angle gradient-echo (FLASH) sequence without slice selection. Our low-field MR system is far from optimized; nevertheless images obtained to date have a 2-D resolution (i.e., pixel size) of ~ 1 mm x 1 mm, and were acquired in ~ 16 seconds.

This research supports the development of new biomedical diagnostic technology — MRI and MRS of laser-polarized noble gases. Large nuclear spin polarizations (> 10%) can be created in dense samples of the spin 1/2 noble gases (^3He and ^{129}Xe) using the technique of spin-exchange optical pumping. Such large polarizations greatly enhance the magnetic resonance detection sensitivity of ^3He and ^{129}Xe , enabling high resolution gas space imaging, studies of gas diffusion in porous and granular media, and investigations of fluids using the soluble ^{129}Xe species. Polarized ^3He and ^{129}Xe can be benignly inhaled by humans with minimal loss of spin-polarization (T_1 ~ 5 to 50 seconds, depending on the organ and tissue) and then detected with MRI/MRS. Potential biomedical applications include improved lung imaging (important for emphysema diagnosis); the imaging of lipid membranes in the brain (useful in the diagnosis of multiple sclerosis and in research on brain function); and better measurement of blood flow to tissue (important for stroke and ischemia diagnosis, and also useful in research on brain function). In addition, noble gas laser-polarization occurs external to the body and does not require a large magnetic field. As a result, at low magnetic fields (~ 0.01 tesla) the SNR of laser-polarized noble gas MRI/MRS can be much larger than the SNR of proton MRI/MRS. Low-field noble gas MRI/MRS may thus be practical in a small, low-power device and enable both portable ground-based and practical space-based biomedical MRI/MRS systems. Our biomedical investigations using polarized noble gas MRI/MRS are in collaboration with the Magnetic Resonance Division of the Brigham and Women's Hospital, headed by Dr. Ferenc Jolesz.

FY97 Publications, Presentations, and Other Accomplishments:

Patent Approved, U.S. Patent #: 5,617,860 Chupp, T.E., Coulter, K., Oteiza, E.R., and Walsworth, R.L. "Method and system for producing polarized ^{129}Xe gas."

Hoffmann, D., Wong, G., Tseng, C.H., and Walsworth, R.L. "Large scale polarization of noble gas for use in medical imaging." American Physical Society Meeting, Washington, DC (April 18 - 21, 1997).

Peled, S., Jolesz, F.A., Tseng, C.H., Nascimben, L., Albert, M.S., and Walsworth, R.L. "Determinants of tissue delivery for ^{129}Xe magnetic resonance in humans." *Magnetic Resonance in Med.*, vol. 36, 340 (1996).

Sakai, K., Bilek, A.M., Oteiza, E., Walsworth, R.L., Balamore, D., Jolesz, F.A., and Albert, M.S. "Temporal dynamics of hyperpolarized ^{129}Xe resonances in living rats." Centre de Physique des Houches International Workshop on Perspectives of MR Imaging Using Polarized Gases, Les Houches, France (October 9 - 11, 1996).

Tseng, C.H., Peled, S., Nascimben, L., Oteiza, E., Walsworth, R.L., and Jolesz, F.A. "NMR of laser-polarized ^{129}Xe in blood foam." *J. Magnetic Resonance*, vol. 126, 79 (1997).

Tseng, C.H., Peled, S., Nascimben, L., Wong, G., Walsworth, R.L., and Jolesz, F.A. "Laser polarized ^{129}Xe spectra and lifetimes in in vitro blood and blood derivatives." Centre de Physique des Houches International Workshop on Perspectives of MR Imaging Using Polarized Gases, Les Houches, France (October 9 - 11, 1996).

Tseng, C.H., Peled, S., Nascimben, L., Wong, G., Walsworth, R.L., and Jolesz, F.A. "NMR of laser polarized ^{129}Xe in blood foam." American Physical Society Meeting, Washington, DC (April 18 - 21, 1997).

Tseng, C.H., Peled, S., Nascimben, L., Wong, G., Walsworth, R.L., and Jolesz, F.A. "NMR of laser polarized ^{129}Xe in blood foam." 1997 Experimental NMR Conference (ENC), Orlando, FL (March 24 - 28, 1997).

Zhao, L., Mulkern, R., Tseng, C.H., Williamson, D., Patz, S., Kraft, R., Walsworth, R.L., Jolesz, F.A., and Albert, M.S. "Gradient echo imaging considerations for hyperpolarized ^{129}Xe MR." *J. Magnetic Resonance*, vol. 113, Series B, 179 (1996).

Zhao, L., Mulkern, R., Tseng, C.H., Williamson, D., Patz, S., Kraft, R., Walsworth, R.L., Jolesz, F.A., and Albert, M.S. "Optimal pulse sequences for hyperpolarized ^{129}Xe MR." Centre de Physique des Houches International Workshop on Perspectives of MR Imaging Using Polarized Gases, Les Houches, France (October 9 - 11, 1996).

Adapting to Altered Gravity and Vision

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Funding:

UPN/Project Identification: 199-16-12-34

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 5

Post-Doctoral Associates: 0

FY 1997 Funding: \$146,000

Responsible NASA Center: Ames Research Center

Task Description:

A series of experiments was undertaken, each aimed at testing and elaborating the hypothesis that repeated alternation between atypical ("rearranged") and normal sensory environments, or between two rearranged sensory environments, leads to the acquisition of a separate adaptations to each ("dual adaptation") and an increased ability to adapt to a novel sensory rearrangement ("adaptive generalization"). The goals of this investigation were to document the ability of human subjects to adapt to the sensory rearrangement in question, to produce dual adaptation, and finally, if the latter occurs, to look for adaptive generalization. The sensory rearrangements examined included pitched visual environments, altered eye-head relationships, and the illusory visual motion that occurs when the relationship between head movements and the location of a visual target is altered.

In one set of experiments, the question was whether and to what extent human subjects would adapt their visual perception to a situation in which the gravitational and visual coordinates were at odds. This unusual visual environment was created by means of a room that was pitched either 20 degrees forward or backward. It was demonstrated that walking back and forth within this room for about 20 minutes caused a reduction in the amount by which the pitched environment biased the observer's apparent visual eye level and also resulted in a post-exposure "negative aftereffect." However, a follow-up study demonstrated that this apparent adaptive change in vision was probably more "cognitive" than perceptual, so this line of research was discontinued.

In a second series of studies, the possibility that the vestibulo-ocular reflex was subject to dual adaptation and adaptive generalization was examined. In Experiment 1 of this series, we found that active exposure to an altered target-head movement relationship led to adaptation and dual adaptation, but not adaptive generalization. Experiment 2 showed that when exposure to the altered target-head relationship was *passively* produced (by means of a rotating chair), only adaptation was obtained.

In a third investigation, we demonstrated that the apparent movement of the visual field that occurs when the head-target relationship is altered (i.e., the loss of visual position constancy) is subject to dual adaptation.

The ability to adapt (and readapt) to altered visual and gravitational-inertial environments has relevance for the rehabilitation of individuals suffering from sensory and motor deficits as, for example, from a stroke or brain damage. Adapting to altered vestibulo-ocular reflexes is assumed to be an important aspect of understanding and overcoming motion sickness, a common malady for riders of Earth-bound vehicles (e.g., ships, planes, cars). The effects of and adaptation to pitched visual environments has direct relevance to understanding and overcoming the problems of balance suffered by individuals who have lost the function of (or were born without) their vestibular organs and, as a consequence, must depend largely on their vision to maintain their balance.

FY97 Publications, Presentations, and Other Accomplishments:

Bridgeman, B., Williams, J.A., and Welch, R.B. "Adaptive generalization and dual adaptation in the human vestibulo-ocular reflex (VOR)." Meetings of the Association for Research on Vision and Ophthalmology (May, 1997).

Clifford, J.O., Tomko, D.L., Sueoka, S.H., and Welch, R.B. (Poster) 37th Annual Meeting of the Psychonomic Society, Chicago (sponsored by Welch) (November, 1996).

Corcoran, M., Daunton, N., Fox, R., Welch, R.B., and Wu, L.-C. "Effects of repeated chronic exposures to 2G on disruption of air righting reflex in the rat." (Poster) Meetings of the ASGSB. Charlotte, NC (October, 1996).

Post, R.B. and Welch, R.B. "Simultaneous opposite adaptations of apparent concomitant motion contingent on the frequency of head motion." *Perception & Psychophysics* (In Press).

Post, R.B., Welch, R.B., and Clark, V.D. "Visually perceived eye level: Effects of pitch perception and eye position." 37th Annual Meeting of the Psychonomic Society, Chicago (November, 1996).

Welch, R.B. "The presence of aftereffects" in "Design of Computing Systems: Cognitive Considerations." Edited by: Salvendy, G., Smith, M.J., and Koubek, R.J. Amsterdam: Elsevier, pp 273-276 (1997).

Welch, R.B., Aratow, M., Ballard, R., Boda, W.I., Hargens, A.R., Watenpugh, D., Rasmussen, D., and Blackmon, T.T. "Adaptation to virtual gravitational environments." (Poster) Ames Director Discretionary Fund poster session (October, 1996).

Welch, R.B., Bridgeman, B., Williams, J.A., and Semmler, R. "Dual adaptation and adaptive generalization of the human vestibulo-ocular reflex." *Perception & Psychophysics* (In Press).

Williams, J.A., Bridgeman, B., Welch, R.B., and Strain, V. "Frequency vs. acceleration specificity in human VOR adaptation." Meetings of the Association for Research on Vision and Ophthalmology (May, 1997).

Skeletal Adaptation to Physical Activity

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Funding:

UPN/Project Identification: 199-26-12-35

Solicitation: 93-OLMSA-07

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Students Funded Under Research: 2

Post-Doctoral Associates: 1

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Responsible NASA Center: Ames Research Center

Task Description:

Exercise has not been entirely successful in maintaining bone mass in cosmonauts during space flight. One reason may be the lack of quantitative data on normal daily activity and exercise from which to develop a basis for selecting and optimizing specific exercises. Experimental studies have identified peak cyclic forces, number of loading cycles, and loading rate as contributors to the regulation of bone density and structure. We have hypothesized that bone density and structure are maintained by daily tissue effective stress histories generated by physical activity. Furthermore, application of these ideas to the calcaneus and lower limbs suggests that bone density and long bone structural integrity in these regions may be quantified in terms of the daily histories of the ground reaction force (GRF). The objectives of this proposal are: (1) to examine the relationship between the spatial distribution of mineral at a long bone cross-section and the cross-sectional flexural properties, i.e., rigidities; (2) to quantify physical activity in terms of the daily history of ground reaction forces; and (3) to examine whether calcaneal bone density and long bone (tibia) cross-sectional areal properties are correlated to the daily history of ground reaction forces. Additionally, we have begun a collaborative effort with the Department of Veterans Affairs (Dr. Gary Beaupré) and Department of Radiology, Stanford University (Dr. Sandy Napel) to develop state-of-the-art surface-based registration algorithms for registration of serial calcaneal scans (partial VA support). (Because of a 1/3 funding cut in proposal budget, a large collaborative third-year human subjects study with Dr. Robert Marcus was postponed.) *Study #1.* Male and female tibiae will be scanned and strain-gaged by our methods. Geometric properties obtained from the scans will be correlated to flexural rigidities obtained from strain gage data. *Study #2.* Daily activity levels of 25 non-exercising subjects will be measured for three days using the GRF system, log books, and pedometers. Calcaneal bone density and long bone properties will be correlated to the mean daily history of the ground reaction force using parameters derived from our model of bone adaptation, pedometers, as well as other functions of daily GRF history obtained from the loggers. Expected results. *Study #1.* Since densitometry measures the distribution of the load-carrying mineral, we expect our results to be highly correlated to measurements computed from *in vitro* surface strain measurements. *Study #2.* We expect the correlation of bone density and structure to activity level to improve with increasingly quantitative measures of physical activity with a best fit using our model and GRF peak cycles and loads from the logger.

The objective of this proposal is to integrate our mathematical model of bone adaptation, novel instrumentation to monitor daily lower limb musculoskeletal loading and new high resolution quantitative computed tomography (QCT) bone imaging methods to study bone loss and adaptation in humans. Our current research, in collaboration with the Palo Alto Veterans Administration and Department of Radiology at Stanford University, is focused on developing the human calcaneus (heel bone) as a model bone site to investigate non-invasively bone adaptation to chronic (spinal cord injury) and acute (space flight) disuse, exercise, drug therapy, and aging. We have chosen the calcaneus because (1) it is loaded by daily ground reaction forces which we can measure; (2) it is a peripheral bone site and therefore more easily and accurately imaged; (3) it is composed primarily of cancellous bone; (4) it is a highly relevant site for monitoring bone loss in astronauts; and (5) it is clinically relevant as the bone of choice for diagnostic ultrasound measurement.

1. Measurement of daily ground reaction forces. Daily ground reaction forces were collected on 24 subjects for a period of three continuous days. Average age was 41 years (min: 27 yr; max: 65 yr). Daily sensor calibrations using a force plate as the gold standard were performed. In addition, the sensor prompts the user every two hours for an in-field zero and one body weight calibration. Sensor data were post-processed to correct for sensor non-linearities. Sensor accuracy was determined to be on the order of ± 0.08 body weights over the applied range of zero to 2.5-3.0 body weights. Histograms of daily loading were computed for each subject. Individual histograms of cycles of daily loading vs. peak cyclic force levels exhibited a characteristic pattern. A considerable range of loading was observed in our group of subjects. We examined high load activity and found that there was a tendency for high load cycles (>2.0 body weights) to decline with age.

2. Registration of CT Images. The accurate measurement of bone apparent density from computed tomography (CT) requires a consideration of image noise, marrow presence and beam hardening. Non-invasive measurement of changes in bone apparent density further necessitates imaging the same skeletal site at different times and registering the serial images. We have developed a semi-automated, 3D, surface-based registration technique that does not require the use of an external frame or fiducial markers. The accuracy of our non-invasive registration technique is comparable to that which can be obtained using standard invasive approaches (e.g., skeletal pins or frames). To test the accuracy and precision of our registration method, we scanned in six different orientations of excised calcanei secured to a custom-made fiducial frame surrounded with water. Technique factors for the GE HiSpeed Advantage CT scanner were: helical mode; 120 kVp; 240 mA; 1.0 mm slice thickness; pitch 1.7; scan field of view 250 mm; display field of view 130 mm. Reconstructions were done every 0.5 mm in the through-plane direction. In-plane pixel size was approximately 0.25 x 0.25 mm. The mean error for all points within the calcaneus from surface-based registration was 0.20 mm (± 0.017). The maximum registration error was 0.40 mm (± 0.11). To our knowledge this whole-bone registration method permits accurate registration of bone volumes an order of magnitude smaller than previously possible using clinical CT scanners.

3. Beam Hardening Correction of CT Images. Beam hardening is caused by the filtering of the polychromatic x-ray source by objects in the scan field of view such as patient fixtures, soft tissue, and bone. If uncorrected, beam hardening can cause severe artifacts in CT images and result in inaccurate measurement of bone apparent density. We have developed an iterative algorithm that eliminates beam hardening error with single energy CT which can be applied to data obtained from any standard clinical scanner. The method requires: 1) objects in the scan be comprised of one or two components only, e.g., cancellous bone comprised of marrow and bone; 2) the attenuation properties of each component to be known as a function of energy; and 3) the x-ray beam intensity vs. energy profile to be known. To test our algorithm, we scanned four concentrations of potassium phosphate in a cow bone cylinder surrounded by water and five calibration tubes. Thirty scans were taken of each slice and averaged to minimize noise in the results. The maximum mean error in the attenuation value of the potassium phosphate (\pm SD) following correction using our method was 1.5% ($\pm 1.5\%$) for the concentration 200 mg/cc K_2HPO_4 . Results from this low noise test produced more accurate and precise measurements of bone equivalent density and at the same time eliminated the need for a calibration phantom.

The primary goal of our research is to clarify the relationship between the musculoskeletal tissue stress (strain) histories developed during normal daily activity and functional adaptation of musculoskeletal tissue. This proposal addresses the both research and technical goals that NASA, the National Research Council, the National

Institute of Health, and the National Institute on Aging (NIA) have identified as critical to space biology and medical science and health care on Earth (in *A Strategy for Space Biology and Medical Science for the 1980s and 1990s*, National Academy Press, 1987; *The Effects of Space Travel on the Musculoskeletal System*, NIH Publ. No. 93-3484). In addition, the NIA has targeted "Frailty" (age-related biomechanical factors affecting physical performance), "Osteoporosis" (non-estrogenic factors affecting bone loss and bone strength), and "Physical Exercise" (effects of exercise on bone and muscle mass and function) as high priority research areas. Specifically, we have hypothesized that bone loss in space and bone loss with age on Earth are in large part due to reduced daily cumulative loading in space and declining activity level with age on Earth. The objective of this research is to integrate our mathematical model of bone adaptation, novel instrumentation to monitor daily lower limb musculoskeletal loading and advanced bone imaging methods using projected radiography (DXA) and quantitative computed tomography (QCT) to study bone adaptation non-invasively in humans. We are focusing on the calcaneus and lower limb bones as model sites loaded by muscles and joint forces that are predominantly determined by the external GRF. These bone sites are also most significantly affected by long duration space flight. We have selected the calcaneus as a primary model bone site because (1) it is loaded by daily ground reaction forces which we now can measure; (2) it is a peripheral bone site and therefore more easily and accurately imaged; (3) it is composed primarily of cancellous bone; (4) it is a highly relevant site for monitoring bone loss in astronauts; and (5) it is clinically relevant as the bone of choice for diagnostic ultrasound measurement. We believe our ground-reaction-force-monitoring instrumentation, bone imaging techniques to examine structural changes in lower limb long bones from planar densitometry and QCT in the calcaneus, developed in collaboration with colleagues at the Palo Alto Veterans Administration and the Department of Radiology at Stanford, represent state-of-the-art contributions to the field. Areas of future applicability include the study of bone adaptation to chronic (spinal cord injury) and acute (space flight) disuse, exercise, drug therapy, and aging.

FY97 Publications, Presentations, and Other Accomplishments:

Breit, G.A. and Whalen, R.T. "Prediction of human gait parameters from temporal measurements of foot-ground contact." *Med. Sci. Sports Exerc.*, 29, 540-547 (1997).

Patent Pending, U. S. Patent #: Undetermined Whalen, R.T. and Breit, G.A. "Method and apparatus for monitoring of daily activity in terms of ground reaction forces."

Yan, C.H., Beaupré, G.S., Whalen, R.T., and Napel, S. "Accurate single energy beam hardening correction for quantitative computed tomography." (Abstract) Proceedings of the 83rd Annual Meeting of the Radiological Society of North America, 205(P)-1050, p. 389 (1997).

Yan, C.H., Beaupré, G.S., Whalen, R.T., and Napel, S. "Registration of serial skeletal images for accurately measuring changes in bone density." (Abstract) Trans of the 43rd Annual Meeting of the Orthopaedic Research Society, Vol 22/section 1: p. 260 (1997).

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Biochemical Changes of Bone in a Model of Weightlessness

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Task Description:

The long-term goals of this research are to understand, on a molecular and biochemical level, the mechanisms of bone structural adaptation observed during space flight and after landing. By analyzing rat bones from two space flight experiments (Cosmos 1887 and 2044), we have recently found that a mineral deficit occurs in some regions of bone and is associated with an alteration of collagen cross-linking. Our underlying hypothesis is that the perturbation of the mineralization process in reaction to weightlessness and upon return is caused by changes of collagen fibrillar structure arranged and stabilized by intermolecular cross-linking. Our continuous research on the characterization of collagen cross-linking and fibrillar structure in the various connective tissues (mineralized as well as nonmineralized, normal as well as pathological) have been and will be the basis for this study. As a simulation of weightlessness, a mature rat model of the one-legged long-term immobilization will be employed. Two groups of adult rats will be prepared for this study: a control (sham) and a group which will be subjected to immobilization (15 weeks) and subsequent reambulation (20 weeks). During the course of this study, bones (femurs) of the following conditions will be studied: 1) normal (control); 2) unloaded and subsequent reambulation; and 3) overloaded and subsequent reambulation. Due to the differences in the turnover rate, the cancellous bone of femur metaphyses and compact bone of diaphyses will be collected separately and subjected to a detailed characterization. The molecular packing structure of type I collagen fibril will be investigated by quantifying the intermolecular cross-links and their precursor aldehydes at their specific molecular loci within the fibril. The analyses of bone mineral will include the content of mineral and its crystallinity measured by electron paramagnetic resonance (EPR) spectroscopy. The mechanical properties of bone will be evaluated to seek any correlation of these properties to the nature of bone mineral and collagen fibrillar structure. These data would provide insight into a regulatory role of collagen structure in the deposit and growth of mineral crystals during bone structural adaptation to various mechanical stresses.

In collaboration with Dr. Grindeland, NASA Ames Research Center, we have performed some biochemical and immunohistochemical analyses on bone matrix using femurs of rats that were subjected to tail suspension for 38 days. Femurs were collected from experimental (suspension, n = 5) and control (ambulatory n = 4). Two bones from each group were fixed, decalcified, and embedded in paraffin. The sections were subjected to

immunohistochemical analysis using monoclonal antibodies, 2B6 (anti chondroitin-4-sulfate), 3B3 (anti chondroitin-6-sulfate) and 5D4 (anti keratan sulfate) and employing the standard immunoperoxidase technique.

The most striking finding was distinct staining pattern for chondroitin-4-sulfate in the subperiosteal bone and within endosteum and bone marrow. In ambulatory bones, the immunopositive areas were well defined and were restricted to endosteum and subperiosteal bone with very little staining within bone marrow. In contrast, in unloaded bones the staining was much more diffuse, particularly within the subperiosteal bone matrix and, to a lesser extent, within endosteum. Interestingly, bone marrow showed strong positive reaction in unloaded bone samples. At this point, it is unclear whether the diffuse staining of bone matrix for chondroitin-4-sulfate is due to the bleaching of the proteoglycan molecules into the matrix from the bone marrow and/or periosteum or an elevated synthesis and/or decreased degradation of chondroitin-4-sulfate in unloaded bones. Immunolocalization of both keratan sulfate and chondroitin-6-sulfate did not reveal any detectable differences between control and suspended bones.

Some bones were pulverized with a Spex Freezer Mill, demineralized with EDTA, washed with deionized distilled water and lyophilized. Approximately 2 mg of bone collagen from suspended ($n = 3$) and control ($n = 2$) were reduced with standardized NaB^3H_4 , hydrolyzed with 6N HCl and subjected to amino acid and cross-link analyses as described previously (Yamauchi et al., 1989). The concentrations were calculated by a mol/mol of collagen basis (SD).

The data showed a clear trend of an increase in labile reducible cross-links as well as deoxy-pyridinoline in suspended bone collagen when compared to those of control. An increase in labile cross-links is indicative of immature nature of collagen fibrils. In addition, the degree of lysine hydroxylation was different between suspended group and control (i.e., lysine-involved cross-links, HLNL and d-Pyr are significantly higher in suspended group). This indicates that newly synthesized collagens during skeletal unloading are post-translationally altered. This alteration could affect the degree of glycosylation of collagen molecules that leads to an altered collagen fibril structure.

Obviously, more analyses are needed to confirm the above preliminary data and characterize these bones in more detail.

Based on the stoichiometry and stereochemistry of intermolecular cross-linking, we have also shown that type I collagen fibrils have more than one molecular packing modality. Since the intermolecular cross-linking is a major determinant of physicomechanical properties of the matrix, these studies could provide an explanation of amazingly diverse functions of connective tissues.

We have also been studying cross-linking chemistries in various pathological bones obtained from osteopetrotic rats, osteogenesis imperfect mice, vitamin B6-deficient chickens, and fibrous dysplasia humans. These comparative characterizations could provide data concerning possible mechanisms of the disordered mineralization.

Our new studies on unloaded bones could provide some insights into the roles of matrix molecules (collagen and proteoglycans) in hypomineralization caused by disuse and/or microgravity.

In collaboration with Drs. Caterson and Lester, we produced and partially characterized monoclonal antibody (1-A-6) raised against the C-terminal derived pyridinoline cross-link peptides isolated from human bone. We already confirmed that the 1-A-6 positive material in human urine contained pyridinoline and deoxypyridinoline cross-link peptides. This could be an excellent diagnostic tool to monitor bone resorption (clinical application). Thus, the Earth benefits derived from this research are multifold from a basic understanding of the collagen fibril structure and mechanisms of bone mineralization and bone loss to a clinical application.

FY97 Publications, Presentations, and Other Accomplishments:

Cheng, H., Caterson, B., and Yamauchi, M. "Biochemical and immunohistochemical analyses of versican in bovine cementum." (Abstract) *J. Dent. Res.*, 76, 267 (1997).

Cheng, H., Caterson, B., and Yamauchi, M. "Chondroitin sulfate proteoglycans in tooth cementum." (Abstract) *J. Dent. Res.*, 75, 261 (1996).

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Seibel, M.J., Baylink, D.J., Farley, J.R., Epstein, S., Yamauchi, M., Eastell, R., Pols, H.A.P., Raisz, L.G., and Gundberg, C.M. "Basic science and clinical utility of biochemical markers of bone turnover." *J. Exp. Clin. Endocrinol. Diabetes*, 105, 125-133 (1997).

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Yamauchi, M., Katz, E.P., Chandler, G.S., Cheng, H., and Crenshaw, M.A. "Distinct post-translational chemistry of mineralized type I collagen." (Abstract) *J. Dent. Res.*, 75, 153 (1996).

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Visual Vestibular Interaction

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Task Description:**Project 1: Human Visual Orientation (C.M. Oman)**

The goal of this project is to better understand how visual scene content influences perception of orientation ("tilt," "location," "direction") and motion ("speed" and "rotation") and conversely, how the perceived orientation of objects influences perceived self-orientation in both real and virtual environments.

Project 2: Subjective Responses to Linear Acceleration and Haptic Stimulation (L.R. Young)

The overall objective of this project is the continuing development of a quantitative and general theory of spatial orientation, expandable to the control of eye, head, and posture control. The emphasis is on linear and angular acceleration stimuli, coupled with optokinetic vection stimuli; we will also increasingly encompass inputs gained from the companion studies on other modalities, specifically, the roles of haptic (tactile and proprioceptive) cues, mental sets, learning and experience, and active versus passive control. Consideration of a rotating device to produce artificial gravity as a countermeasure has become a major new direction for our research and has already involved a human factors (movement control) and a cardiovascular response experiment.

Project 3: Human Factors and Physiological Effects of Short-Arm Centrifugation

This project is designed to determine appropriate methods of applying artificial gravity to astronauts. Before a short-arm centrifuge or spinning vehicle can be tested in space, ground studies must be conducted to determine the physiological effects of a force gradient and the human factors issues associated with rotation. Thus, our project has two components: human factors and gravitational physiology. The purpose of the human factors portion of the study is to determine if the performance of two-handed tasks is affected by Coriolis forces. The second portion of this investigation focuses on determining several of the cardiovascular effects of a force gradient on a normal human subject. Studies are conducted through the use of the MIT-Artificial Gravity Simulator (AGS), essentially a two m-radius, rotating platform, in the MIT Man-Vehicle Laboratory.

In Project 1, our prototype helmet mounted Virtual Environment display system was used in three different experiments. The first was a study of tilt, vection, and visual reorientation illusions experienced by subjects

inside a virtual room which slowly tumbles about them. The visual reorientation illusions are similar in character to those experienced by astronauts. Latency and saturation of vection depended strongly on scene content and symmetry, but only weakly on posture. Reorientation illusions occurred at consistent phase angles which differed between subjects. The second experiment studied the latency and magnitude of linear vection produced by a looming virtual stimulus. Independent variables included field of view, stimulus speed, orientation with respect to gravity, and adaptation/learning effects. The third is a study of the interaction of tactile, visual, and linear acceleration cues, described under Project 2 below. Script-driven VR experiment control software originally developed for this project has evolved into an Experiment Manager now being used in the NASA JSC Virtual Environment Generator on the Neurolab Shuttle Mission.

Three studies comprised Project 2. The first study used the sled facility to investigate the hypothesis that otolith information may be used for recreating a path in space. Blindfolded subjects were passively accelerated along a horizontal track and instructed to indicate when they passed a target. Three orientations were used: Y axis (interaural), +Z axis (rostro-caudal, headward), and -Z axis (rostro-caudal, footward). We found that responses were significantly more anticipatory for footward acceleration than for headward acceleration.

The second study investigated the effect of adaptation to weightlessness on ocular counter-rolling (OCR) induced by static tilt. OCR was measured pre- and post-flight in four (4) astronauts on the SLS-2 mission. We found that in three of the four subjects, the magnitude of OCR was reduced post-flight. This finding lends support to the otolith tilt-translation reinterpretation hypothesis. In addition, all four subjects demonstrated changes in the left-right symmetry of OCR following flight. These results bear on both neurovestibular compensation and space motion sickness.

The third study investigated the role of tactile information in linear motion perception, using the sled facility and the NASA Langley G-seat. An experiment was conducted in which subjects were given control of the sled in rostro-caudal axis horizontal motion and presented with sum-of-sines disturbances in sled velocity and G-seat pressure. Their task was to null out sled velocity. Estimator transfer functions were calculated for the vestibular and tactile channel. Results showed significant control responses to G-seat pressure, characterized by lead-lag transfer functions, for at least 8 of 12 frequencies in 7 of 8 subjects. The lead-lag character of the tactile channel estimator transfer function supports the use of the G-seat as an acceleration onset cueing device.

Project 3 focused on determining several of the cardiovascular effects of a gravity gradient on normal subjects. The study has investigated the following questions: 1) how cardiovascular performance measures change with G level and duration of stimulation, 2) how cardiovascular parameters change during force gradient stimulation as compared to their response to standing in 1 G, and 3) what levels of force gradient stimulation promote significant cardiovascular regulation. It was hypothesized that G levels of 1 and less at the feet would produce few cardiovascular changes in normal subjects. This investigation offers data that future researchers can use to more effectively design the centrifuge studies necessary on individuals undergoing bed rest treatments as models for space flight deconditioning.

Eight subjects, four men and four women, participated in one control and three rotation trials on a horizontal short-arm centrifuge (SAC) such that the G_z levels at the feet were 0.5, 1.0, and 1.5. Trials consisted of 30 minutes of supine rest, 1 hour of rotation (or in the control, 30 additional minutes of rest and 30 minutes of standing), and a final 30-minute rest period. Measurements of heart rate, calf impedance, calf volume, and blood pressure were obtained. Post-trial analysis explored the relationships between the physical characteristics of the subjects, rotation time, G level, and the cardiovascular parameters measured. Most of the measured cardiac parameters suggest that rotation levels causing 1.0 G or less at the feet produced regulatory responses not significantly different from continued supine rest. In addition, cardiovascular responses to SAC rotation with 1.5 G at the feet were statistically similar to standing, at least for a comparison based on 30 minutes. The primary effects of 1.5 G were an elevated diastolic pressure, increased heart rate, and increased calf volume. While some cardiovascular changes were found to be correlated to gender, mass, and height, their influence was considered minor. Since bed rest trials show that standing decreases orthostatic intolerance, and our experiments suggest that rotation at 1.5 G produces effects that mimic those found in a subject standing erect, short-arm

centrifugation should be seriously considered as a possible countermeasure to cardiovascular space deconditioning. Rotation durations of approximately 30 minutes might be required for promotion of sufficient cardiovascular regulation in space, where a SAC may someday be used to keep the cardiovascular system stimulated to minimize orthostatic intolerance.

This project has a great deal to contribute to the neuro-otology community. The otolith organs are gravity sensors and are uniquely affected by the weightlessness of orbital flight. The sorts of problems created for astronauts and the ways of dealing with neurovestibular adaptation to space flight and return to Earth are highly relevant to neuro-otology and otolaryngology. The ability to "turn off" the constant pull of gravity in space provides the vestibular physiologist with an ideal tool for study of the basic function of the vestibular apparatus in spatial orientation and motor control. The process of overcoming the disturbing disorientation and space sickness associated with space travel should bear on the process of vestibular rehabilitation of patients on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

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Growth Regulation in the Adult Cardiac Muscle Cell

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Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Task Description:

This grant is based on the hypothesis that normal cardiac structure, composition, and function are the direct and dynamic ongoing result of a normal myocardial loading environment, not a fixed property of cardiac tissue. Deviations above or below this normal loading set point cause abnormalities in each of these myocardial properties. This hypothesis applies equally to increases and decreases in cardiac load. Astronauts exposed to microgravity (decreased load) for greater than seven days develop a decrease in cardiac mass (atrophy). Patients with long-standing pressure or volume overload (increased load) develop an increase in cardiac mass (hypertrophy). Whether mechanisms which control an increase in mass are equal and opposite to those which control a decrease in mass is unknown; however, it is likely that insights into one process will aid understanding of both of the possible mechanisms causing alterations in cardiac mass include a change in myocardial load or a change in neurohumoral activation. Importantly, these two potential mechanisms may be complementary rather than alternative. Attempts to examine these mechanisms have been limited by the complexities of *in vivo* experiments, where it is difficult to completely separate changes in load from changes in neurohormones. An alternate approach would be to study growth regulation in primary cell culture. To date, however, methods have not been developed which allow adult mammalian cardiac muscle cells (cardiocytes) to be maintained in long-term primary culture in mitogen-free medium, with no extensive changes in phenotype and with preserved mechanical and electrical function. Thus, the specific aims of this grant are to: 1) develop methods by which adult cardiocytes can be maintained in long-term culture and be induced by alterations in mechanical load to have a graded increase or decrease in cell mass; 2) determine the relative importance of load changes versus changes in neurohormones in altering growth regulation; and 3) determine the mechanisms by which load alterations are transduced into changes in cell mass. Preliminary studies suggested that cardiocyte mass could be decreased, increased, or maintained unchanged in long-term culture using electric field stimulated contraction and specific culture methods. These changes in cardiocyte mass appeared to coordinate with changes in protein synthesis rate. Based on these studies, we designed protocols in which cardiocytes will be embedded in an agarose matrix, perfused with medium, electrically stimulated to contract, and maintained in culture for 7-14 days. Graded alterations in the major determinants of load: stimulation frequency, tension development, cardiocyte length, and the tension-time index will be imposed on cardiocytes in long-term culture. As the percent agarose is increased, the matrix becomes stiffer, cardiocyte contraction becomes more nearly isometric, and cardiocytes develop more tension. As the agarose is stretched, cells embedded in the agarose will be stretched to longer cell lengths.

Sequential effects of these protocols on cardiocyte morphology, function, mass, and protein synthesis, and the mechanisms affecting these changes in growth regulation will be examined. These studies will define the primary dynamic regulators of the structural and functional properties of adult myocardium.

Specific Aim #1: "Develop methods to maintain adult cardiac muscle cells in long-term primary culture with preserved normal phenotype and normal contractile function." This specific aim has been partially completed. We developed a method to maintain adult cardiac muscle cells in long-term primary culture using a specific and well defined media with cells cultured on laminin multiwell trays. Using this model cardiocytes maintain normal phenotype and normal contractile function. These methods are described in a manuscript entitled "Growth Effects of Electrically Stimulated Contraction on Adult Feline Cardiocytes in Primary Culture," *Am. J. Physiol.* 1995, 268: H2495 - H2504.

We have begun to culture adult cardiocytes in a matrix using either agarose or type I collagen. We have performed short-term studies which examined protein synthesis rates in cells embedded for 24 hours and electrically stimulated. Culture for longer term will require development of methods which allow adequate diffusion of buffer through the gel in volumes large enough to maintain normal electrolyte and nutrient levels.

Specific Aim #2: "Develop methods to induce a graded decrease or increase in cardiac muscle cell mass by imposing an alteration and mechanical load on adult cardiac muscle cells maintained in primary culture. Determine the effects of these changes on cell mass and cardiocyte mechanical function." This specific aim has been partially completed. Using the models outlined under Specific Aim #1 combined with electrical stimulation, cardiac muscle cell mass increased over a 7 day period of time in part because there was a significant increase in protein synthesis rate. These methods are described in the manuscript cited above.

Specific Aim #3: "Determine whether alterations in cell load (stress) or cell length (strain) contribute to changes in muscle cell mass." We have begun looking at the effects of changes in cell load (stress) by embedding cardiocytes in variable concentrations of type I collagen. To date we have done preliminary studies showing that protein synthesis rate increases in parallel with an increase in the percent collagen used to make the gel from 1% to 16%. As the percentage of collagen in the gel is increased, the gel stiffness increases and the impediment to shortening increases causing the cardiocytes to contract more isometrically and with more force. The increasing impediment to shortening has been documented using an edge detection system to measure cell shortening extent and velocity. To prove that this decrease in shortening was caused by an increased load rather than a nonspecific injury to cells, contraction was examined in cells embedded in collagen and then in the same cells after the collagen matrix was removed by treatment with collagenase. After removal of collagen, contraction returned to normal.

Specific Aim #4: "Determine whether neurohormones of the sympathetic nervous system or the renin angiotensin aldosterone system alter growth regulation in adult cardiac muscle cells." Using the model outlined under Specific Aim #1, we examined the effect of angiotensin II on protein synthesis rate and cardiac muscle cell growth during seven days of culture with and without electrical stimulation. Angiotensin II caused a moderate growth effect but did not augment growth in the presence of electrical stimulation. In addition, angiotensin AT1 receptor blockade with Losartin or AT2 receptor blockade with PD123319 did not inhibit the growth effect of electrical stimulation. In these studies angiotensin II binding studies were performed to examine the affinity and number of angiotensin II receptors in each of the above protocols. None of these protocols affected angiotensin II binding. These experiments are described in a manuscript entitled "Comparative Effects of Contraction and Angiotensin II on Growth of Adult Feline Cardiocytes in Primary Culture," *Am. J. Physiol.*, 271: H29-H37, 1996.

Therefore, we have accomplished substantial portions of specific aims 1, 2, 3, and 4 during the past academic year.

This grant is based on the central hypothesis that changes in hemodynamic load and/or changes in neurohormonal activation are the primary dynamic regulators of the structural and functional properties of adult myocardium. To date, studies suggest that normal cardiac structure, composition, and function are the direct and

dynamic result of a normal myocardial loading environment and are not a fixed property of the tissue. Myocardial load can be influenced by alterations in stress (force produced by the myocardium during contraction) or strain (change in myocardial length produced by the application of a force). When load is normal, myocardial structure, composition, and function are also normal. However, deviations above or below this normal loading set-point cause abnormalities in each of these three properties of the myocardium. For example, a decrease in load causes atrophy as evidenced by a decrease in mass, cardiocyte cross-sectional area (CSA), and myofibrillar volume, and a decreased contractile state, as evidenced by a decrease in the force-velocity relationship. In contrast, an increase in load causes hypertrophy with an increased mass, CSA, and myofibrillar volume and decreased contractile state, with an increase in the force velocity relationship. These changes are rapid (two weeks) and pronounced. Importantly, if these abnormalities are not excessive in degree or duration, they are totally reversible and do not result in a fixed pathological defect. When load returns to normal, myocardial structure, composition, and function return to normal. These data led us to further hypothesize that there is a spectrum of cardiac properties which are defined by a spectrum of cardiac loading conditions and that the mid-point of this loading spectrum results in normal cardiac structure, composition, and function. Therefore, proving or disproving this hypothesis will help us identify the mechanisms responsible for two important phenomena: first, the changes in cardiac structure, composition, and function which occur during manned space flight in microgravity, where hemodynamic load is reduced; and second, the changes which result from cardiac disease in man, where hemodynamic load is increased. Studies described in the grant apply equally to studies of cardiac unloading in microgravity with resultant atrophy and studies of cardiac overloading in disease with resultant hypertrophy. In particular, this grant will: 1) examine processes attendant to a decrease in cardiac mass (atrophy); 2) provide a model which can be used to extend studies of atrophy and hypertrophy to adult cardiocytes maintained in long-term culture in which alterations in mechanical load can be used to induce a graded increase or decrease in cell mass; 3) determine the relative importance of changes in load or changes in neurohormones in altering growth regulation; and 4) define the mechanisms by which alterations in load are transduced and translated into changes in cell mass. Furthermore, this work will help to define the mechanisms responsible for the changes in myocardial structure, composition, and function which result both from microgravity, where a decreased load causes atrophy, and cardiovascular disease in normal gravity, where an increased load causes hypertrophy. Once these mechanisms have been identified, new treatments can be developed to alter or prevent the clinical consequences of atrophy and hypertrophy.

FY97 Publications, Presentations, and Other Accomplishments:

Hamawaki, M., Coffman, T.M., Lashus, A., Koide, M., Zile, M.R., Oliverio, M.I., Defreyte, G., Cooper, G., and Carabello, B.A. "Pressure overload hypertrophy is unabated in mice devoid of AT1A receptors." *Am. J. Physiol.* (In Press).

Kato, S., Koide, M., Cooper, G., and Zile, M.R. "Effects of pressure or volume overload hypertrophy on passive stiffness in isolated adult cardiac muscle cells." *Am. J. Physiol.*, 271, H2575-2583 (1996).

Koide, M., Carabello, B.A., Conrad, C.C., Buckley, J.M., Defreyte, G., Barnes, M., Tomonek, R., Cooper, G., and Zile, M.R. "The effect of losartan on the extent of right ventricular pressure overload hypertrophy and muscle dysfunction." *Am. J. Physiol.* (In Press).

Koide, M., Carabello, B.A., Conrad, C.H., Tomanek, R.J., Cooper, G., and Zile, M.R. "The effect of angiotensin receptor blockade on the mass and function of pressure overload hypertrophy." (Abstract) *Circulation*, 94 [Suppl. I], 429 (1996).

Koide, M., Nagatsu, M., Zile, M.R., DeFreyte, G., Tagawa, H., Cooper, G., and Carabello, B.A. "Premorbid determinants of left ventricular dysfunction in a novel model of gradually induced pressure overload in the adult canine." *Circulation*, 95, 1601-1610 (1997).

Matsuo, T., Carabello, B.A., Zile, M.R., Koide, M., and McDermott, P.J. "Mechanisms of cardiac hypertrophy in canine volume overload." *Am. J. Physiol.* (In Press).

- Tagawa, H., Koide, M., Sato, H., Zile, M.R., Carabello, B.A., and Cooper, G. "Cytoskeletal role in the transition from compensated to decompensated hypertrophy during adult canine left ventricular pressure overloading." *Circ. Res.* (In Press).
- Tagawa, H., Wang, N., Narishige, T., Ingber, D.E., Zile, M.R., and Cooper, G. "Cytoskeletal mechanics in pressure overload cardiac hypertrophy." *Circ. Res.*, 80, 281-289 (1997).
- Tanaka, R., Barnes, M.A., Cooper, G., and Zile, M.R. "Effects of anisotonic stress on cardiac muscle cell length, diameter, area, and sarcomere length." *Am. J. Physiol.*, 270, H1414-1422 (1996).
- Wada, H., Zile, M.R., Ivester, C., and McDermott, P.J. "Comparative effects of electrically stimulated contraction and angiotensin II on growth of adult feline cardiocytes in long-term primary culture." (Abstract) *J. Am. Coll. Cardiol.*, 27 [Suppl A], 27A (1996).
- Wada, H., Zile, M.R., Ivester, C.T., Cooper, G., and McDermott, P.J. "Comparative effects of contraction and angiotensin II on growth of adult feline cardiocytes in primary culture." *Am. J. Physiol.*, 271, H29-H37 (1996).
- Zile, M.R., Bennett, L.C., Barnes, M.A., and Cooper, G. "Effects of alterations in viscous damping on the cardiocyte force versus frequency relationship." (Abstract) *Circulation* (In Press).
- Zile, M.R., Cowles, M.K., Buckley, J.M., Richardson, K., Cowles, B.A., Cooper, G., and Gharpuray, V. "Gel stretch method: A new method to measure constitutive properties of cardiac muscle cells." *Am. J. Physiol.* (In Press).
- Zile, M.R., Gharpuray, V., Cowles, M., and Cooper, G. "Constitutive properties of pressure hypertrophied cardiocytes." (Abstract) *Circulation*, 94 [Suppl. I], 547 (1996).
- Zile, M.R., Koide, M., Sato, H., Conrad, C.C., Buckley, J.M., and Cooper, G. "Role of microtubules in the contractile dysfunction of hypertrophied myocardium." *Cardiovasc. Res.* (In Press).
- Zile, M.R., Richardson, K., Buckley, J.M., Koide, M., Cowles, M.K., Cowles, B.A., Gharpuray, V., and Cooper, G. "Constitutive properties of adult mammalian cardiac muscle cells." *Circulation* (In Press).

Mechanical and Molecular Stimuli for Normalizing Muscle Mass During Unloading

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Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 4

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 166,962

Task Description:

Unloading of postural skeletal muscle, as seen during space flight or ground-based models such as bedrest or limb suspension, may result in loss of muscle mass and changes in myosin heavy chain (MHC) phenotype. A series of studies are proposed that would more clearly define the primary mechanical stimuli required for altering fiber size and inducing transformations in MHC phenotype. The proposed studies will impose precisely controlled contractile training paradigms ranging from protocols which optimize power output to those which result in near maximal force production on single muscles in the unloaded (via tail suspension) hindlimbs of rats. These training paradigms will be evaluated with respect to various parameters (e.g., mass, MHC type, total protein and RNA content) to identify the best candidates for an exercise countermeasures program. To investigate the involvement of insulin like growth factor 1 (IGF-I) in the mediation of muscle mass conservation, the levels of IGF-I protein, mRNA, and receptors will be measured. Later phase experiments will directly manipulate IGF-I levels in a single target muscle during chronic unloading with or without training.

Research Aim - Elucidation of the role of IGF-I in the maintenance of muscle mass.

Our previous results showed that overloaded skeletal muscles increase their production of insulin-like growth factor-I (IGF-I) and that this increase precedes overt evidence of a hypertrophy response. This indicated that autocrine/ paracrine IGF-I production by overloaded skeletal muscles demonstrated the appropriate temporal relationship to be considered a mediator of this process. In these studies we found that the muscle IGF-I response to increased loading was relatively independent of the circulating levels of hormones and growth factors known to regulate skeletal muscle mass. We also reported that changes in IGF-I are correlated with changes in total muscle DNA. Further investigations have shown that this increase in DNA is a result of satellite cell proliferation. *In vitro* studies by a number of investigators have demonstrated that IGF-I is the only well characterized growth factor which can stimulate muscle cells to proliferate and to differentiate (i.e., to express muscle specific proteins).

In light of our findings and *in vitro* results from the literature, we have postulated that one potential mechanism for the local mediation of skeletal muscle hypertrophy involves the production of IGF-I by overloaded myofibers leading to anabolic responses and the activation of satellite cells which first proliferate then differentiate and fuse with existing myofibers to provide additional myonuclei. In further support of this hypothesis, recent studies

conducted by other investigators have demonstrated that these cell proliferation and differentiation processes are required for muscles to enlarge in response to increased loading.

Based on these findings, we designed a series of experiments to test this hypothesis. The experimental model we employed involved the implantation of osmotic pumps which delivered various growth factors directly into the myofibrillar compartment of individual skeletal muscles in intact rats. In a manuscript currently in revision, we report that infusion of non-systemic doses of IGF-I and to a lesser extent Growth Hormone (GH) results in skeletal muscle hypertrophy. Another growth factor, fibroblast growth factor-2 (FGF-2), did not induce muscle hypertrophy. In both the IGF-I and GH infused skeletal muscles, apparent satellite cell proliferation resulted in the maintenance of a constant proportionality between muscle DNA and muscle protein content. However, FGF-2 resulted in an increase in muscle DNA while no increase in protein occurred. Since GH is known to increase muscle IGF-I production, these findings appear to support the hypothesis that IGF-I can stimulate skeletal muscle hypertrophy processes *in vivo*. FGF-2 has been shown to stimulate satellite cell proliferation *in vitro*, but is antagonistic to the process of cell differentiation. Therefore, our recent results suggest that overload induced IGF-I production by myofibers may mediate skeletal muscle hypertrophy by stimulating anabolic metabolism and by activating satellite cells to provide additional myonuclei thereby maintaining the proportion of DNA to protein in affected muscles.

Loss of muscle mass, termed muscle atrophy, is associated with numerous myopathies as well as unloading due to confinement, casting, and space travel. The ability to prevent muscle atrophy and the attendant loss of function would have obvious and extensive application. Insulin-Like Growth Factor-1 (IGF-I) has been shown, in cell culture models, to increase muscle cell protein production. IGF-I is being actively studied for a variety of therapeutic uses, many of which are related to the maintenance of muscle mass and function. The studies being conducted as part of this grant speak to the understanding of the fundamental relationships between muscle IGF-I production and the maintenance of muscle mass. Understanding of these basic biological relationships is critical for the development of advanced therapeutic strategies for the prevention of muscle atrophy.

FY97 Publications, Presentations, and Other Accomplishments:

Adams, G.R. "The role of IGF-I in muscle adaptation" in "Exercise and Sport Science Reviews." Edited by: Holloszy, J.O. Williams & Wilkins, Baltimore, MD (In Press).

Adams, G.R. "Muscle hypertrophy resulting from direct IGF-I infusion." *Med. Sci. Sports Exercise*, 29(5):S114 (1997).

Adams, G.R. and Haddad, F. "The relationships among IGF-I, DNA content and protein accumulation during skeletal muscle hypertrophy." *J. Appl. Physiol.*, 81(6), 2509-2516 (1996).

Transduction Mechanisms in Vestibular Otolith Hair Cells

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-40-17-06

Solicitation: 12-10&11-92/GB

Initial Funding Date: 1995

Expiration: 1995

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: not available

Task Description:

We have recently shown that hair cells in the bullfrog vestibular otolith organs possess different complements of membrane conductances. We now wish to study how these conductances, which modify the responses of hair cells to natural stimulation, are acquired and regulated during normal development. Using the aminoglycoside antibiotic gentamicin sulfate to induce the regeneration of selective hair cell populations, we will study the morphological and physiological development of regenerating hair cells in explant cultures of the vestibular otolith organs.

Using immunocytochemical methods to label mitotically active cells, we will identify the progenitor cell(s) giving rise to new hair cells, compare the rates of on-going and gentamicin-induced cell proliferation, and determine at what developmental times regenerating hair cell types can be identified by their cell or hair bundle morphology. We will also study, using video microscopy, the morphogenesis of individual regenerating hair cells in wholemount cultures.

We will isolate regenerating hair cells from organ cultures and, using whole-cell patch-clamp techniques, determine their responses to intracellular current. We will then determine the time of appearance of specific membrane currents in regenerating hair cells and study changes in the size and gating kinetics of their underlying membrane conductances at different developmental times. We will also examine the importance of specific membrane currents for hair cell development by blocking these currents with selective antagonists and observing the subsequent morphological and physiological development of regenerating hair cells. If possible, immunocytochemical methods will be used to confirm the existence of specific ion channel proteins in regenerating hair cells.

This project was completed during early FY97.

We are investigating how receptor hair cells in the peripheral vestibular apparatus transduce mechanical displacement into electrical signals. These studies are important for understanding the operation of the vestibular endorgans in normal and pathological conditions and for understanding how damage to the vestibular endorgans affects body coordination. The vertebrate saccular and utricular maculae transduce the linear forces produced by static head displacement relative to gravity and by dynamic translational head acceleration into neural signals. Saccular and utricular hair cells with differing hair bundle morphology differ in their voltage-dependent

conductances. These conductances, by acting as frequency-dependent filters of the receptor current, modify the sensitivity and frequency selectivity of hair cells. Utricular hair cells also differ in their rate of adaptation to sustained head displacement. Nonadapting hair cells are most sensitive to static gravity and adapting hair cells, because they do not retain information about maintained displacement, are most sensitive to changes in linear acceleration. The dual encoding functions of the vestibular otolith organs are therefore largely accomplished by varying the rate and extent of adaptation in different hair cell phenotypes.

Hair cells in the bullfrog vestibular otolith organs regenerate following aminoglycoside ototoxicity. Hair cells in these organs are differentially sensitive to gentamicin, with saccular hair cells and hair cells in the utricular striola being damaged at lower gentamicin concentrations than hair cells in the utricular extrastriola. Regenerating hair cells in these organs have short hair bundles and can be classified into a number of phenotypes using the same morphological criteria used to identify their mature counterparts. BrdU-labeling studies in living animals and *in vitro* organ cultures indicate that hair cell recovery in the vestibular otolith organs is accomplished by both mitotic and non-mitotic mechanisms. The former mechanism is known to produce hair cells through the mitotic division of precursor cells. Our studies also suggest that some supporting cells can convert, or transdifferentiate, into hair cells without an intervening cell division. By stimulating one or both of these processes in humans, clinicians may be able to alleviate human deafness and peripheral vestibular disorders through the direct replacement of lost hair cells.

FY97 Publications, Presentations, and Other Accomplishments:

Baird, R.A., Steyger, P.S., and Schuff, N.R. "Intracellular distributions and punitive functions of calcium-binding proteins in the bullfrog vestibular otolith organs." *Hear. Res.*, 103, 85-100 (1997).

Steyger, P.S., Burton, M.D., Hawkins, J.R., Schuff, N.R., and Baird, R.A. "Calcium-binding proteins are early markers of non-mitotically regenerating vestibular otolith hair cells." *Int. J. Develop. Neurosci.*, 15, No. 4/5, 417-432 (1997).

Steyger, P.S., Gillespie, P.G., and Baird, R.A. "Myosin 1b is located at tip-link anchors in adapting and non-adapting vestibular hair bundles." (Abstract) *Assoc. Res. Otolaryngol.*, 20 (1997).

Effect of Skeletal Unloading on Bone Formation

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Funding:

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Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$190,345

Task Description:

Skeletal unloading results in a transient decrease in bone growth associated with a decrease in osteoblast number. The bone becomes less mineralized, and osteocalcin levels fall; at the molecular level, the ratio of alkaline phosphatase to osteocalcin mRNA levels increases. These observations support the concept that the osteoblast population is depleted during skeletal unloading in part by a block in pre-osteoblast recruitment, and in part by a decrease in committed osteoblast proliferation and differentiation. Administration of growth hormone (GH) or insulin-like growth factor-1 (IGF-I), the local factor through which GH exerts at least some of its growth promoting effects on bone, fails to reverse the inhibition of bone formation except at supraphysiologic doses. The IGF-I mRNA levels increase during skeletal unloading suggesting that IGF-I production is not inhibited. These changes in bone in response to skeletal unloading suggest the hypothesis that the decrease in bone formation induced by skeletal unloading, a consequence in part of resistance to the growth promoting effects of growth hormone, results from a decline in the osteoprogenitor stem cell population, a decrease in osteoblastic progenitor cell proliferation, and differentiation into mature osteoblasts. To test this hypothesis, we propose to determine the effect of skeletal unloading on the osteogenic stem cell population, osteoblastic progenitor recruitment and osteoblastic proliferation, and differentiation *in vivo* and *in vitro*. This will be accomplished by evaluating the number of osteoblast and stromal cells from loaded and unloaded bones capable of developing into bone cell forming colonies, their proliferative activity, and their rate of differentiation as assessed by the sequential expression of c-fos, type I collagen, alkaline phosphatase, and osteocalcin. We will then determine the effect of skeletal unloading on the ability of GH to regulate bone cell growth and differentiation by assessing the proliferative and prodifferentiating response of osteoblasts and stromal cells to GH and IGF-I *in vivo* and *in vitro*. Finally, we will determine the ability of cell loading to stimulate bone cell responsiveness to GH and IGF-I using both the Flexercell system and low speed centrifugation to load the cells and then evaluate the effects of loading on cell proliferation and differentiation. We expect these experiments to provide insight into the mechanism(s) by which skeletal unloading leads to inhibited bone formation.

During the past several years, our laboratory has focused on the role of IGF-I as an important link between skeletal unloading and bone formation. We established that IGF-I and II were both important in the fetal development of bone, but that IGF-I played the dominant role in post-natal life of the rat. In the younger rats (6 wk old), IGF-I mRNA and protein levels actually increase with skeletal unloading. This was a surprise. These

levels remained elevated during the 2 week recovery period during which bone formation rose above normal. Similar results were found in bones from space flight animals, confirming the paradox that the factor we expected to mediate bone formation in response to skeletal unloading actually rose while bone formation fell. To confirm these observations and to identify where in bone the IGF-I mRNA and protein levels were affected by unloading, we performed *in situ* hybridization to examine the changes in mRNA and immunolocalization. Because our previous studies indicated that 6 wk old rats had the lowest levels of IGF-I mRNA, we evaluated the effects of skeletal unloading (5d) on IGF-I mRNA and protein levels in 3 wk and 10 wk old animals. IGF-I mRNA was localized primarily to the growth plate within the proliferative and hypertrophic zones as well as the cells lining the trabeculae of the primary and secondary spongiosa. Immunolocalization of IGF-I protein followed a similar pattern. Grain counts from the cells in nine different sections of the growth plates of two animals per group were tabulated. The results indicate that skeletal unloading does not alter the distribution of either the mRNA or the protein. The much larger hypertrophic cells of the growth plate contained 3 to 4 times as much IGF-I mRNA as the smaller proliferating cells, although the intensity of labeling was higher in the proliferative zone. Skeletal unloading did not alter this ratio and had no significant effect on IGF-I mRNA levels by this method of quantitation. Thus we were unable to confirm the rise in IGF-I mRNA seen with analysis of total RNA from bone, but the results indicate that the fall in bone formation in the proximal tibia with skeletal unloading is not due to a substantial decrease in IGF-I mRNA.

The lack of correlation between IGF-I production and bone formation during skeletal unloading was explained when we infused IGF-I into control and hindlimb elevated rats and discovered that the hindlimb elevated rats were refractory to the bone growth promoting effects of IGF-I.

To confirm the resistance to IGF-I established in the preceding experiments, we examined the ability of GH to reverse or prevent the inhibition of bone formation induced by skeletal unloading. These experiments were performed in hypophysectomized (HPX) rats unlike the preceding experiments with IGF-I. GH has its own receptors in bone and activates a number of pathways, but its major effect on bone formation is thought to be through IGF-I. We demonstrated that GH increases IGF-I mRNA levels equally well in both loaded and unloaded bones. Furthermore, GH increased the mRNA levels for a number of bone formation markers such as osteocalcin, alkaline phosphatase, and collagen. In all cases, both the shaft and metaphysis responded to GH with a comparable rise in the aforementioned mRNAs in both loaded and unloaded bones. However, bone formation per se at the tibiofibular junction and the tibial metaphysis showed a markedly blunted response to GH in the hindlimb-elevated animals. These data suggest that the unloaded bone responds normally to GH at the level of IGF-I induction and the induction of the genes required for bone formation, but the full pathway leading to a differentiated osteoblast capable of producing mineralized bone is defective.

To exclude the possibility that the resistance to IGF-I and GH reflects a general resistance to all anabolic hormones, we evaluated the effect of skeletal unloading on the response to PTH. Bone formation varies along the shaft of the tibia, and differs whether the periosteal surface or endosteal surface is examined. Furthermore, skeletal unloading decreases bone formation in the periosteum substantially more than in the endosteum. However, the ability of PTH to stimulate bone formation was not altered by skeletal unloading either in the tibial shaft or in the metaphysis. These results are consistent with our observation that the PTH receptor mRNA and protein are not altered by skeletal unloading at least at the growth plate. Like GH, PTH stimulates the mRNA levels of osteocalcin comparably in both loaded and unloaded bone, but unlike GH, PTH stimulates osteocalcin mRNA much more in the metaphysis than shaft, whereas GH is effective in both regions.

The site selective effects of skeletal unloading and anabolic hormones on bone formation indicate that more precise localization of the bone formation markers will be required to understand the full impact of these perturbations on bone. Consequently, we have developed *in situ* hybridization and histolocalization methods for identifying the mRNA and protein levels of these markers within bone. The markers studied to date include osteocalcin, alkaline phosphatase, and collagen. Osteocalcin mRNA is found primarily in the hypertrophic cells of the growth plate, within osteoblasts in the secondary spongiosa lining the trabeculae, and within osteoblasts lining the endocortical surface of the proximal cortex. Little mRNA for osteocalcin is seen in the periosteal cells. Skeletal unloading for 5d did not have a substantial effect on either the distribution or the amount of

osteocalcin mRNA observed. These results are not inconsistent with Northern analyses demonstrating a modest and transient reduction of osteocalcin mRNA levels during skeletal unloading, a decrease that would be difficult to confirm with the less quantitative *in situ* hybridization methodology. Osteocalcin protein was distributed to the same cells as its mRNA, and, likewise, showed no major alteration in distribution or quantity with skeletal unloading. Alkaline phosphatase mRNA, on the other hand, is increased by skeletal unloading using Northern analysis, an initially surprising result but which we believe reflects retarded differentiation of the osteoblast induced by skeletal unloading. *In situ* hybridization demonstrates alkaline phosphatase mRNA in the hypertrophic chondrocytes of the growth plate and in osteoblasts lining the trabeculae of the metaphysis. Little alkaline phosphatase mRNA was seen in the cells lining the endosteal or periosteal surfaces of the cortex. Skeletal unloading increased the amount of alkaline phosphatase mRNA in the growth plate and metaphysis without altering its distribution. When the distribution of alkaline phosphatase activity was assessed, the activity appeared to be greatest within the trabecular and cortical bone rather than in the osteoblasts lining the trabeculae and cortex. Like the mRNA levels, alkaline phosphatase activity appeared to be increased by skeletal unloading. The mRNA for the $\alpha 1$ chain of type 1 collagen is found primarily in the osteoblasts of the primary and secondary spongiosa and those cells lining the endosteal surface. Little mRNA for collagen is seen in the periosteal cells of the proximal tibia, but as one looks more distally along the shaft, the amount of collagen mRNA in periosteal cells increases while the levels in the endosteal cells decrease. These results indicate that of the markers examined, collagen mRNA levels best parallel bone formation rates. Skeletal unloading results in a substantial reduction in collagen mRNA levels in these areas, a result which confirms the reported decrease in collagen mRNA during skeletal unloading when assessed by Northern analysis. Thus, the *in situ* hybridization results in general confirm our previous observations using RNA extracted from bone, but provide the additional capacity to determine whether the bone cells are responding in a site selective fashion to skeletal unloading and growth promoting agents like GH and IGF-I.

Although skeletal unloading is likely to alter the activity of existing osteoblasts, as suggested by the changes in mRNA levels for the bone formation markers in those cells, skeletal unloading may also alter the differentiation of osteoprogenitor cells into mature osteoblasts. To address this possibility, we isolated the osteoprogenitor cells from the marrow of the tibia of hindlimb elevated (five days) and control animals. After 5 days of culture, these cells were assessed for proliferation using ^3H -thymidine incorporation and cell number using crystal violet staining. On a per cell basis, the cells from the unloaded tibia had a 50% reduction in ^3H -thymidine incorporation (623 ± 45 cpm vs 1219 ± 255 cpm for unloaded and loaded, respectively). We then examined the differentiation pattern of these cells in culture. Competitive RT-PCR was used to determine the mRNA levels for IGF-I, c-fos, and alkaline phosphatase in cells as they differentiated in culture. The mRNA for IGF-I was maximal by 5 to 7 days of culture, then fell reaching a nadir at 10 days, but increased again over the subsequent two weeks. C-Fos mRNA reached its peak at day 10, with a rapid fall thereafter. Alkaline phosphatase mRNA levels peaked at day 15 before gradually falling. Osteocalcin (BGP) mRNA levels were the last to rise, with a peak on day 20. Alkaline phosphatase activity was measured in these same cells and was found to increase rapidly between day 10 to 15, lagging the rise in mRNA levels by several days. We then examined the effects of skeletal unloading on this pattern. In this experiment, the marrow osteoprogenitor cells were obtained from the tibiae of control animals and animals hindlimb elevated for 2 or 5 days. The cells were grown in 10mM β -glycerophosphate from the time of plating which reduced their growth rate compared to the previously described experiment in which a lower concentration of β -glycerophosphate was used. This shifted (delayed) the time course without altering the pattern. C-Fos mRNA peaked at 15d in this experiment, and was reduced 50% in the cells from the unloaded tibiae (both 2d and 5d unloaded bones). Alkaline phosphatase mRNA in the cells from the control tibiae peaked at 20d in culture, but this peak was higher and occurred earlier in the cells from the unloaded bones. In this case, the effect was more striking in cells from the bones of the 5d hindlimb-elevated animals than in those from the 2d hindlimb-elevated animals. The levels of BGP mRNA remained low throughout this experiment, and did not peak even by 28d. Nevertheless, skeletal unloading reduced these levels further at all time points examined. As a final differentiation marker we assessed calcification of the nodules formed by 28d. The cells from the unloaded (5d) bones showed a 40% reduction in calcification as assessed by alizarin red staining. Overall, these data suggest that the alterations in cell proliferation and differentiation induced by skeletal unloading *in vivo* persist when the osteoprogenitor cells are cultured *in vitro*. The persistence of the effects of skeletal unloading on proliferation and differentiation of the

osteoprogenitor cells during culture permits us to study the mechanisms *in vitro*, a substantial advantage for a number of assays we intend to perform.

In an effort to establish an additional model by which the resistance to IGF-I induced by unloading could be studied *in vitro*, we examined the effect of mechanical strain on IGF-I stimulated alkaline phosphatase activity of osteoprogenitor cells in culture. Marrow osteoprogenitor cells were plated into dishes with collagen coated flexible bottoms. After 2d, half of the cultures were placed in a Flexercell apparatus and subjected to a maximum of 2% elongation at 0.1Hz for 8h/day for 4d. During the final 24h, the cultures were treated with 100ng/ml IGF-I or vehicle then harvested for alkaline phosphatase and protein determination. In this experiment, mechanical strain by itself had little effect on alkaline phosphatase activity, and IGF-I in the absence of mechanical strain increased alkaline phosphatase levels only 30%. However, the combination of mechanical strain plus IGF-I led to a 101% increase in alkaline phosphatase activity. These promising preliminary experiments are being expanded to examine the interaction between IGF-I and mechanical strain on a variety of proliferation and differentiation markers. However, the level of strain induced by the Flexercell may be above physiologic levels even at the lowest elongation level (2%) that we can achieve with consistency. Therefore, we have established a collaboration with Dr. Tony Keaveny at UC Berkeley to assist in constructing a pulsed fluid flow apparatus to provide what may be a more physiologic approach to applying a mechanical load on these bone cells.

Loss of bone is a major problem for animals and humans undergoing microgravity for extended lengths of time. The return of humans from space flight is accompanied by increased risk of fracture. At this point, it is not clear that the bone lost is ever fully regained. Bone loss during space flight is not the only clinical condition that is addressed by this project, however. Humans immobilized by disease also lose bone. Unlike astronauts who are healthy and with normal skeletons at the time of space flight, patients immobilized for extended periods of time are often already deficient in bone mass such that acute losses during immobilization put such individuals at a high risk of fracture and deformity. Efforts to determine the mechanism by which immobilization or microgravity leads to bone loss should result in the design of rational drug therapy to prevent or reverse the loss.

FY97 Publications, Presentations, and Other Accomplishments:

Bikle, D.D. "Biochemical markers in the assessment of bone disease." *Am. J. Med.* (In Press).

Bikle, D.D., Halloran, B.P., and Morey-Holton, E. "Space flight and the skeleton: Lessons for the earthbound." *Endocrinologist*, 7, 10-22 (1997).

Halloran, B.P., Bikle, D.D., Harris, J., Tanner, S., Curran, T., and Morey-Holton, E. "Regional responsiveness of the tibia to intermittent administration of parathyroid hormone as affected by skeletal unloading." *J. Bone Min. Res.*, 12, 1068-1074 (1997).

Kostenuik, P.J., Halloran, B.P., Morey-Holton, E.R., and Bikle, D.D. "Skeletal unloading alters the *in vitro* differentiation of rat osteoprogenitor cells." *Am. J. Physiol.* (In Press).

Otolith-Canal Convergence in Vestibular Nuclei Neurons

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Post-Doctoral Associates: 0

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Task Description:

During manned space flight, acute vestibular disturbances often occur, leading to physical duress and a loss of performance. Vestibular adaptation to the weightless environment follows within two to three days, yet the mechanisms responsible for the disturbance and subsequent adaptation are still unknown. The current investigation is determining how the vestibular nuclei neurons quantitatively synthesize afferent information from the different linear and angular acceleration receptors in the vestibular labyrinths into an integrated output signal. Since information from the vestibular nuclei is presented to different brain regions associated with differing reflexive and sensory functions, it is important to understand the computational mechanisms used by vestibular neurons to produce the appropriate output signal. Utilizing linear translation, rotational motion, and the unique advantages offered by a mechanical stimulation technique developed in our laboratory, the effects of convergence of information from linear to angular acceleration receptors onto single vestibular nuclei neurons will be determined.

Before meaningful data could be interpreted regarding the responses of vestibular nuclei neurons to convergence of semicircular canal and otolith inputs, knowledge regarding the response properties of vestibular primary afferents was needed. Thus, studies were initiated to determine the spatial orientations of the semicircular canals and their innervating afferents in pigeons. It was found that afferents of the three semicircular canals had directions of maximum sensitivity to rotational stimulation that were orthogonally arranged in three-dimensional space. Afferents specific to one canal had directional rotation vectors that were tightly clustered together indicating that all afferents for individual canals had similar spatial properties. The orthogonality between the directions of rotation vectors for the different canal afferents was interesting for several reasons. First, rotational head accelerations are signaled by the across fiber pattern response from all afferents, where the response gain for individual afferents varies as a cosine function of the angle between rotation direction and the orientation of the canal plane. Thus, when rotations are directed within the major plane of one canal, the responses from its innervating afferents are maximal, while responses from afferents innervating the other canals are minimal. Second, the orthogonality in the rotation vectors of afferents innervating the three canals is important, since deviations from the orthogonality between the major anatomical planes of the bony canals were found. These findings are described in further detail in a paper published from this study (Dickman, 1996).

Next, a similar investigation was undertaken to determine both the spatial and temporal response properties of otolith afferents innervating the utricle in pigeons. The spatial properties of otolith afferents were examined

using linear acceleration stimuli in the horizontal plane where directions of maximal sensitivity were determined for each recorded utricular fiber. It was found that the polarization vectors were scattered throughout the horizontal plane, with the majority of vectors being directed toward the contralateral ear. The breadth of tuning of the otolith afferents was also studied and it was found that nearly all fibers were cosine tuned. Finally, the response dynamics of the afferents were studied using different frequencies of sinusoidal linear translations. Increasing gains and constant phase values as a function of stimulus frequency was characteristic for these afferents with simple transfer functions fit to each unit. These results are more thoroughly described in a paper published by Si et al., 1997.

In addition to afferent responses, we wished to know the nature of the eye movement sensitivity of the central vestibular neurons that received convergent canal and otolith information. Thus, three-dimensional eye movement responses (VOR) elicited by off-vertical axis rotations (OVAR) and linear motion were recorded in awake head fixed pigeons using a calibrated dual search coil. Linear translations (0.1 - 0.4g; 0/16 - 10 Hz) and OVAR (0.4g; 8 - 108 deg/sec; 0/02 - 3 Hz) were delivered in the dark to stimulate the otolith system. During low frequency OVAR stimulation, all slow phase eye velocity response component gains were small, but increased as stimulus velocity increased. During linear translations directed perpendicular to the optic axis, mostly torsional eye movements were produced that increased as a function of stimulus frequency. The horizontal response gains were small. During linear motion parallel to the optic axis, primarily vertical eye movements were obtained. For all orientations, the eye movements to linear motion were limited to static and low frequency stimulation, with no responses being obtained to frequencies greater than 1 - 2 Hz. In contrast to frontal eyed animals such as monkeys or cats, the linear VOR in pigeons is limited to only responses relative to gravity tilts and not to high frequency linear accelerations.

Since the initiation of the project in June 1992, Dr. Dickman has been principally engaged in the afferent physiology and eye movement studies described above. Recordings from vestibular nuclei neurons to both linear and angular acceleration stimuli are in progress. The goal will be to determine how these central neurons encode directional movement to both rotational and linear movements. Since, during space flight, the largest linear acceleration stimulus, gravity, is nearly eliminated, it is important to understand how the central vestibular neurons will be affected.

In all vertebrate animals, the vestibular system forms an essential component in the production of movement related responses that are critical for the daily function and survival of the animal. During manned space flight, acute vestibular disturbances frequently occur, with approximately 70% of the shuttle flight crew personnel experiencing symptoms of disorientation, nausea, and emetic attacks during the first 48 - 72 hours of weightlessness. Although a number of investigators have postulated that the lack of gravity as a constant vestibular stimulus during space flight produces profound changes in central nervous system processing of vestibular information, the basic physiological mechanisms of information synthesis by vestibular brainstem neurons in weightlessness or a normal gravity environment is not currently understood. There are, however, several recent reports indicating that the vestibular system is affected by exposure to space flight conditions, with elicited changes in the physiology of vestibular afferent responses and vestibular induced eye movements. The current proposed project will provide answers to the questions regarding the nature of signal processing by gravity sensing mechanisms in vertebrates and their control in movement related reflexes. This information is crucial to form the basis upon which an understanding of the neural sensorimotor adaptations to space flight conditions can be acquired.

FY97 Publications, Presentations, and Other Accomplishments:

Dickman, J.D. "Spatial orientation of the semicircular canals and their innervating afferents in pigeons." *Exp. Brain Res.*, 111, 8-20 (1996).

Dickman, J.D., Beyer, M., Newlands, D.D., McHenry, M.Q., Hess, B.J.M., and Angelaki, D.E. "Linear vestibulo-ocular responses in pigeons." Ninth European Conference on Eye Movements, Ulm, Germany (September, 1997).

Si, X., Angelake, D.E., and Dickman, J.D. "Response properties of pigeon otolith afferents to linear acceleration." *Exp. Brain Res.* (In Press).

Microgravity In Vitro Model of Bone: Flow Effects

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Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 133,620

Task Description:

In physiological bone maintenance and remodeling, the local mechanical environment regulates bone cell turnover such that skeletal architecture is both metabolically and mechanically optimized. Prolonged unloading associated with microgravity alters the dynamics of bone turnover, resulting in complications from skeletal resorption. It has become increasingly evident that a rapid and substantial flow of interstitial fluid occurs across the cortex of bone. Interstitial fluid is driven radially outward across the cortex by the pressure gradient between the vasculature and the lymphatic drainage system at the periosteal surface. Localized compression of porous and elastic bone tissue from mechanical loading also generates transcortical interstitial fluid flow (ISF). The overall objective of this study is to characterize the flow-mediated mechanochemical transduction mechanism(s) in osteoblasts. We will focus on the role of nitric oxide (NO), G-proteins, and cytoskeletal components/membrane fluidity. NO is a powerful inhibitor of osteoclast activity and has dose dependent effects on osteoblast proliferation. We have shown that flow is a potent stimulus of NO in bone, but to date, little is known about the functional nitric oxide synthase (NOS) isoform that is active in bone. Using Western blot techniques and various NOS-specific inhibitors, we will identify the flow-sensitive NOS isoform present in osteoblasts. We will further characterize flow-mediated NO responses by differentiating between shear stress and shear rate sensitivity. In an effort to identify the earliest signaling events in this biochemical pathway, we will also investigate the interaction between NO and prostaglandin E₂ in flow-induced responses. Our preliminary data suggests a role for G-proteins in osteoblast mechanosensitivity, and we will elucidate both the time course and specific G-protein subunit involvement in this pathway. Likewise, cytoskeletal components and membrane fluidity may modulate flow responses. We will independently alter these parameters using chemical agents to stabilize/disrupt these components and monitor flow-induced NO production. Fluid flow is a potent stimulus in bone maintenance and remodeling and therefore both flow and the downstream flow-sensitive biochemical effectors represent a novel approach to treatment strategies for disuse osteoporosis associated with exposure to microgravity.

In Vivo Studies

Over the course of the development of an *in vitro* model of bone, our studies have demonstrated that flow-induced fluid shear results in rapid stimulation of two autocrine and paracrine mediators of bone remodeling, prostaglandin E₂ (PGE₂) and NO. To complement this work, our research within the past year has focused on

the investigation of flow effects *in vivo* using an animal model of altered interstitial fluid flow through bone. Ultimately, our objective is to determine the role of flow perfusion of bone in osteoporosis of disuse/microgravity-induced osteoporosis and to determine the mechanism of flow effects. These goals are dependent on experiments utilizing the rat model that we have developed. Briefly, interstitial fluid flow in one hindlimb is altered by blocking venous return. The buildup of arterial flow causes an increase in the marrow pressure, which in turn causes an increase in the interstitial fluid flow driven by the pressure gradient.

In our initial studies, venous return was blocked by one of three methods. For one group, an air-pressurized cuff designed and built in our laboratory was applied for thirty minutes daily, causing intermittent increases in intraosseous pressure. In the second group, suture ligation of the femoral vein resulted in an increase in intraosseous pressure which was maintained for the duration of the experiment. Partial venous occlusion of the femoral vein was performed in the third group, causing a slight increase in intraosseous pressure. All three of these methods resulted in an increase in the mass and length of the pressurized femurs relative to the contralateral (control) femur, strongly suggesting that alterations in interstitial fluid flow do indeed influence bone remodeling. The mechanism of these effects was investigated in experiments designed to determine the roles of NO and PGE₂ by the administration of the nitric oxide inhibitor aminoguanidine or the cyclooxygenase inhibitor indomethacin. The results of these drug studies, however, were inconclusive; the effects of the drug, administered once daily by intraperitoneal injection, inevitably wore off after a short period. Histomorphometric analysis of bone sections was also performed to further assess bone remodeling in these experiments; here the results were also inconclusive as we determined that one bone section could not be representative of the remodeling along the entire length of the femur. We are modifying both the drug studies and analysis techniques for future experiments.

The results of these initial studies warranted the investigation of the protective effect of altered interstitial fluid flow against bone loss in animal models of osteoporosis; the ultimate objective, again, would be to determine the mechanism of such an effect. We conducted several studies early this year incorporating our model of altered flow in ovariectomized rats, animal models of age-related osteoporosis. More recently, however, we have been investigating the effect of altered flow in an animal model of microgravity-induced osteoporosis, the hindlimb suspended rat. The hindlimb suspended rat has been used extensively as a model for simulation of weightlessness in space flight in which bone atrophy of the unloaded hindlimbs has been observed. This model has even greater value in our research as it is a model independent of mechanical strain. This may prove to be invaluable in attributing any protective effect directly to fluid flow. Interestingly, in our initial hindlimb suspension studies, there appears to be a reversal of bone loss caused by venous ligation. Using dual energy X-ray absorptiometry (DEXA), bone mineral content and bone mineral density were assessed for sections of femur and tibia at the beginning and end of the experiment. Increases in bone mineral content of the ligated leg (+9.5% and +4.8% for the femur and tibia, respectively) were observed, while decreases were noted for the control leg (-3.2% and -8.2%). Similarly, increases in bone mineral density of the ligated leg (+2.2% and +5.0%) were greater than the changes in the control leg (-0.003% and +1.0%). These results suggest that ISF flow can directly influence bone remodeling independently of mechanical loading and supports our hypothesis that fluid flow modulates bone remodeling.

***In Vitro* Studies**

During the past year the main goal of our *in vitro* osteoclast study was to observe the effect of fluid flow-induced shear stress on precursor osteoclasts and osteoclasts. Additionally, we were interested in determining which calcitropic factors were either induced or inhibited by osteoclasts in response to fluid flow. Much of our research during this period centered on the development of techniques to harvest osteoclasts for use in our fluid flow experiments. Osteoclasts are relatively difficult to concentrate due to their fragility, scarcity, and tendency to adhere, but we have been able to overcome these obstacles with techniques designed in our laboratory, including the use of echestratin, FCS-free media, and vitronectin-coated petri dishes.

Using these methods, we were able to obtain a sizable population of precursor osteoclasts. Experiments were then performed to investigate the effect of fluid flow-induced shear stress on these harvested precursor osteoclasts.

In these studies, we reported that fluid flow-induced shear stress (12 dynes/cm²) stimulated a 21-fold increase in PGE₂ release (35 ng/mg/hr) and a 2.25-fold increase in NO release as indicated by both chemiluminiscent nitrate reduction of persulfate (4.7 nmol/mg/hr) and an increase in cGMP activity. We have submitted an abstract of these findings to the American Society of Bone and Mineral Research. In a later experiment, we observed that flow-induced NO production in pre-osteoclasts was inhibited in the presence of L-NAA (100 μM). Taken together, these results suggest that fluid flow directly influences osteoclast function through an autocrine mechanism.

Future Studies

For our *in vivo* studies, we are currently carrying our new hindlimb suspension experiments to reinforce our findings. After these experiments, we will proceed to drug studies investigating the mechanism of flow effects. The modification in the drug study that we plan to introduce is the use of implantable osmotic pumps which will allow for steady, continuous delivery of the drugs. Bone remodeling will be assessed not only with the current methods of direct bone measurement (wet weight and dimensions), histomorphometry, and bone densitometry (DEXA), but also with comparisons of high resolution X-ray to assess shape change, and quite possibly with the use of a CT scanner to assess trabecular bone changes. Changes in trabecular bone may be assessed with other traditional techniques. Related studies which we will pursue in the following year will include the plotting of the relationship between marrow pressure over time (to determine the extent and length of the effects of venous ligation on intraosseous pressure), as well as a comparative study of the effects between static pressure changes and pulsatile pressure changes. *In vitro* studies demonstrate that a single stimulus of fluid shear results in a release of NO that gradually levels off, while consecutive pulses of fluid shear result in continually ramping levels of NO. Examination of this activity *in vivo* may provide new direction for our research.

The next step in our *in vitro* osteoclast study will involve isolating larger and purer populations of viable osteoclasts cells for use in fluid flow experiments. The successful harvesting of such cells will facilitate our investigation of NO production, PGE₂ production, cGMP production, the inhibitory effect of L-NAA, and the inhibitory effect of GDP-BS in osteoclasts. In future studies, we plan to investigate the mechanism of osteoclastogenesis and determine the manner in which precursor osteoclasts fuse to become multinucleated osteoclasts. We would also like to conduct experiments which will allow us to isolate and identify the Nitric Oxide Synthase isoform present in osteoclasts.

FY97 Publications, Presentations, and Other Accomplishments:

Hillsley, M.V. and Frangos, J.A. "Alkaline phosphatase in osteoblasts is down-regulated by pulsatile fluid flow." *Calcif. Tiss. Int.*, 60, 48-53 (1997).

Hillsley, M.V. and Frangos, J.A. "Osteoblast hydraulic conductivity is regulated by calcitonin and parathyroid hormone." *J. Bone Min. Res.*, 11, 114-124 (1996).

Johnson, D.L., McAllister, T.N., and Frangos, J.A. "Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts." *J. Bone Min. Res.*, 271, E205-E208 (1996).

Reich, K.M., McAllister, T.N., Gudi, S.R.P., and Frangos, J.A. "Activation of G proteins mediates flow-induced prostaglandin E2 production in osteoblasts." *Endocrinology*, 138, 1014-1018 (1997).

Regulation of Vesicular Neurosecretion by Mechanical Stress on Integrins in Nerve Terminal Membranes

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

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Task Description:

Sensitivity to subtle mechanical stimuli is critical to the function of many cell types and, in ensemble, to behavior of the organism. This basic research proposal is aimed at understanding a novel and potent form of mechanotransduction mediated by integrins, ubiquitous membrane spanning adhesive molecules implicated in many forms of signaling, mostly via second messenger pathways. We have shown that small changes in mechanical or osmotic stress on integrin-extracellular matrix bonds can strongly modulate neurosecretion from motor nerve terminals. This modulation is extremely rapid and non-adapting, does not require Ca^{++} influx, but is dependent on intracellular Ca^{++} . Immunohistochemical, pharmacological, and electrophysiological techniques will be used to ask what kind(s) of integrins are involved, where they are located, what molecules they interact with, and their mechanism(s) of action. Experiments will discriminate between different hypotheses for the mechanism(s) of modulation of vesicle release, i.e., does tension on integrins evoke release of Ca^{++} from internal stores, or is it independent of changes in internal Ca^{++} ? If the former, what is the source of the Ca^{++} ? If the latter, is the change in physiology due to second messenger mediated phosphorylation/dephosphorylation reactions, or is it a direct (diffusion-limited) mechanical effect on vesicle docking or release reactions in the cell, via the cytoskeleton or other connections? Preliminary data favor the last hypothesis.

Integrins are already known to be among the most important cell adhesion and signal transducing molecules. If they can transduce small mechanical signals into profound changes in cell physiology by direct mechanical means not involving second messenger pathways, this would represent not only a new role for integrins, but a new category of regulatory or mechanosensory mechanism. This might be widely applicable in different cells and tissues, wherever mechanical stimuli are important to function.

Progress has been made on three fronts:

(1) The integrin-mediated modulation of neurotransmitter release has now been shown convincingly to be mechanically mediated and not to involve second messenger signalling within the nerve terminal. Not only is the modulation linearly related to length, with virtually no delay, no phasic component, and no hysteresis, but the temperature co-efficient (Q_{10}) is approximately one, suggesting that the modulation does not depend on chemical reactions. There apparently is a molecular link between the cytoplasmic domain of the integrins and

some component of the release apparatus that, with mechanical stress, pulls two reactive molecules closer together or pulls neurotransmitter-containing vesicles closer to the fusion apparatus in the plasma membrane.

(2) Although the effect of tension on integrins is mechanically mediated, we have found that some critical component of the mechanical link must be in the dephosphorylated state. Okadaic acid, a potent phosphatase inhibitor, eliminates the modulation, while this effect is blocked by staurosporine, a broad spectrum kinase inhibitor. We do not yet know the identity of the protein(s) involved.

(3) The enhancement of neurotransmitter release by hypertonicity, which causes terminals to shrink, has been shown to also involve integrins at least in part. This probably reflects the same mechanism as that mediating stretch modulation of release. In addition, however, hypertonic solutions affect release in other ways not mediated by integrins.

This research is aimed at understanding mechanical signal transduction across cell membranes, the role(s) of integrins in this transduction, and the molecular interrelationships within the sub-plasma membrane cytoskeleton and between the cytoskeleton and the extracellular matrix or other cells. In the particular context of our research, this will have implications for the molecular interactions regulating vesicle docking, priming, and secretion. In the broader context, it should provide useful new information about the role of integrins and mediating the effects of mechanical stimuli on all aspects of cell biology.

FY97 Publications, Presentations, and Other Accomplishments:

Chen, B.M. and Grinnell, A.D. "Effects of phosphatase and kinase inhibitors on the integrin-mediated mechanical modulation of transmitter release from frog motor nerve terminals." *Soc. Neurosci. Abstr.*, 23, 363 (1997).

Chen, B.M. and Grinnell, A.D. "Kinetics, Ca^{2+} dependence, and biophysical properties of integrin-mediated mechanical modulation of transmitter release from frog motor nerve terminals." *J. Neurosci.*, 17, 904-916 (1997).

"Baby Machine" Analysis of Cellular Gravity Sensitivity

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Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$101,011

Task Description:

A newly-developed culture system for mammalian cells, called the "baby machine," has properties ideally suited for studies on the direct effects of gravity on cell growth and division. The advantage of the system is that the cells can be oriented with respect to the gravity vector in the absence of additional external constraints such as the cell-substratum and cell-cell interactions. This culture system will enable ground-based assessments of gravity-sensitive "windows" for any cell process. In this proposal, gravitational effects on mitosis, the cell cycle, the segregation of components between daughter cells, and cellular senescence will be evaluated. Growth and division of the cell cultures will be analyzed with respect to fixed gravity vectors, and during gravity averaging in a clinostat. The involvement of the gravity vector in the orientation of mitosis will be determined, as well as the existence of gravity-sensitive "windows" during the mitotic process. The effects of gravity compensation on mitosis will also be assessed by comparing baby machine-cultured cells maintained in a horizontal-axis clinostat with appropriate controls.

The "baby machine" culture system has been used to investigate gravitational effects on cell division orientations under conditions that mimic autonomous cell growth in suspension culture as nearly as possible. To achieve this aim, Chinese hamster ovary (CHO) cells were attached to tiny adhesive sites, smaller in diameter than the cells, distributed over a nonadhesive coating on the bottom of a polystyrene culture flask. The adhesive sites consisted of 4.8 μm -diameter Dynabeads (Dyna, Inc.). To produce the nonadhesive surface, the bottom of the dish was coated with a 5% solution of poly-2-hydroxyethyl methacrylate (polyHEMA, Sigma) in absolute methanol. CHO cells, in RPMI 1640 medium containing 10% fetal calf serum, were added to the flasks, and they attached only to the beads. The flasks were positioned vertically, and the growth and division of the attached cells were recorded by time-lapse videotaping. The video recordings were used to observe the growth properties of the cells. Division angles were measured for each cell as the direction of the spindle axis at late anaphase in photographs of individual video frames. Division angles were measured for over 500 cells, and the direction of division was found to be random with respect to gravity. Many of the cells divided multiple times during the observations, so that the angles of successive divisions for individual cells could be determined. Consecutive division angles always formed a regular pattern, with equal probability that an attached cell would divide in the same axis or in a perpendicular axis. Thus, gravity did not affect the orientation of mitosis. Perhaps the most important finding from this aspect of the study is that successive divisions of the somatic cells were oriented in orthogonal planes. The choice of plane appeared to be random, unlike the early cleavages of embryos that follow a fixed sequence of perpendicular cleavages. Thus, if the position of the division axis is a

consequence of the movement of centrosomes to opposite sides of the nucleus to establish the locations of the spindle ends, this movement can take place in the same plane, rather than in an orthogonal plane, in two consecutive cell cycles. It was evident from the videotapes that the cells undulated during growth while attached to the beads. Nevertheless, the precise pattern of successive divisions indicates that once the cells attached they remained fixed and did not twist in the vertical plane, perhaps due to the linkage between the adhesion sites and the cytoskeleton. When the cells entered mitosis, there was little further movement, and they seemed to reach a fixed position with respect to the bead, and remained there through metaphase and anaphase.

The next step in this project was to modify the culture system to permit long-term analyses of gravity effects and gravity averaging on cell growth properties, including effects on the segregation of components between sister cells formed at division, and on cellular aging. To accomplish this task, it was necessary to modify the culture system so that one of the two progeny cells is shed from the attachment site at each division, such that the one that remains is always the same when the two can be differentiated. This modification permits continuous production of newborn cells for cell cycle studies on undisturbed cell populations. It also enables analysis of signal distribution between the daughter cells, and identification of changes that take place as cells age or senesce. To achieve this goal, methodology has been developed to hold the cells to the surface by a small pressure differential. The apparatus consists of a thin nickel disc containing 2 μm -diameter holes spaced in an ordered array. The discs were produced by a microlithographic process. The discs were placed in a filter holder and cells were drawn to the holes by a small pressure differential, ca. 0.5 psi. The cells, either CHO or mouse L1210, were held in place on the discs for extended periods, with each hole containing a single bound cell. At division, only one of the two progeny cells remained held to the surface due to the small relative size of the holes. This modified technique is currently being used to examine the effects of long-term incubation on division orientations, and cell cycle parameters. Preliminary results indicate that long-term growth is easily achieved, that successive divisions continue to be in orthogonal planes, that cell cycle parameters change with growth time, and that gravity had little detectable influence on these cell properties. It is anticipated that this new culture system, once fully developed, will simplify many studies on the mammalian cell cycle and the effects of differing cell environments on cell cycle properties. In the next year it will be used to evaluate gravity effects, and gravity averaging, on the cell cycle properties of cells "aging" in very long-term culture.

The purpose of the research is to gain basic information on the effects of gravity on fundamental properties of cell growth, and the manner in which microgravity might influence cell growth processes. The unique aspect of the work is that these issues can be addressed in an easily-performed and very informative ground-based study. It is important to learn whether any cellular process is directly influenced by, determined by, or even dependent on, the presence of gravity. The proposed studies will answer several aspects of these basic questions. If it is found that altered gravity has deleterious effects on aspects of cellular metabolism, then these findings would need to be considered when planning human activities in microgravity environments. Understanding of the basic aspects of cellular gravity sensitivity could then be used to develop remedies for the potential adverse biological responses. Conversely, the current study may identify positive influences of altered gravity on cellular processes which could then be used for the benefit of man on Earth or in microgravity, such as the treatment of diseases which rely on improved growth of normal cells and/or altered growth of diseased cells. In principle, any gravity-sensitive aspect of cell growth detected in this project has the potential to be useful in the design of improved environments for many human activities. In addition, a new cell culture system is being developed in this project which should greatly simplify basic research on the cell cycle, cell development, and cell aging in biology laboratories world-wide.

FY97 Publications, Presentations, and Other Accomplishments:

Helmstetter, C.E. "Gravity and the orientation of cell division." *Proc. Natl. Acad. Sci., USA*, 94:10195-10198 (1997).

Mechanotransduction Through the Cytoskeleton

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Funding:

UPN/Project Identification: 199-40-17-14

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$180,289

Task Description:

The goal of this project is to characterize the molecular mechanism by which cells recognize and respond to physical forces in their local environment. The project is based on the working hypothesis that cells sense mechanical stresses through their cell surface adhesion receptors, such as integrins, and that they respond as a result of structural arrangements with their internal cytoskeleton (CSK) which are orchestrated through use of tensegrity architecture. Work completed and published in the past funding period has provided direct support for this hypothesis. We therefore continue our ongoing studies to define the architectural and molecular basis of cellular mechanotransduction. The new specific aims are to: 1) define the molecular pathway that mediates mechanical force transfer between integrins and the CSK in cells that lack or contain the focal adhesion protein, vinculin; 2) develop a computer simulation of how mechanical stresses alter CSK structure and test this model in living cells; and 3) determine how mechanical deformation of integrin-vinculin-CSK linkages is transduced into a biochemical response. This last aim is based on our past finding that many signal transducing molecules are immobilized on the CSK at the site of integrin binding within the focal adhesion complex and preliminary data which suggest that mechanically stressing these complexes using magnetic twisting cytometry can modulate signal transduction. Elucidation of the mechanism by which cells sense mechanical stresses through integrins and translate them into a biochemical response should help us to understand the molecular basis of the cellular response to gravity as well as many other forms of mechanosensation and tissue regulation.

Over the past grant funding period, we have demonstrated that the focal adhesion protein, vinculin, plays a key role in the mechanical coupling of integrins to the cytoskeleton. Specifically, we demonstrated that mouse F9 carcinoma 5.51 cells that lack vinculin protein spread poorly on extracellular matrix, fail to form actin stress fibers, and exhibit greatly enhanced flexibility when their cell surface integrin receptors are mechanically stressed using magnetic twisting cytometry. In contrast, when vinculin protein was replaced in these cells by transfection, transmembrane mechanical coupling, stress fiber formation, and cell spreading were all restored to near normal levels. These results suggest that vinculin may play a key role in mediating mechanical signal transfer across the cell surface.

Using a micromanipulation approach, we demonstrated that integrins, cytoskeletal filaments, and nuclear scaffolds are mechanically coupled in living cells such that pulling on integrins with micropipettes results in coordinated realignment of structures inside the cytoskeleton and nucleus. Mechanical continuity also was

demonstrated within the nucleus in interphase and mitotic cells using a similar approach. These results confirm that living cells are literally hard-wired by a series of molecular struts and cables that stretch from specific adhesion receptors on the cell surface to discrete attachment sites on the nucleus at the center of the cell, as predicted by the tensional integrity ("tensegrity") model. More recently, we have carried out computer simulations which depict how actomyosin filament nets restructure themselves when mechanical stress balances are altered in living cells. We also incorporated microtubules within these simulations to demonstrate how the pull of the contractile nets causes microtubule buckling to occur as well as how the presence of microtubules alters the mechanics of the actin lattice.

Finally, we have started to map out a mechanical signaling pathway that begins with application of a shear stress to integrins on the cell surface and ends with activation of gene expression in endothelial cells.

In this project, we address the general problem of how animals perceive gravity by focusing on a more specific question: How is a mechanical stimulus transmitted across the cell surface and transduced into a biochemical response within individual cells? Our working hypothesis is that mechanical forces may be transmitted to cells as a result of binding interactions between extracellular matrix attachment molecules and specific transmembrane receptors on the cell surface, such as integrins. Transduction into biochemical information and changes in gene expression would then occur as a result of subsequent alterations in cytoskeletal structure and mechanics inside the cell. This proposal is based upon the observation that cell shape and thus, the form of the cytoskeleton, depends upon a dynamic equilibrium between tensile forces that are generated within contractile microfilaments and resisted both by internal structural elements and by matrix attachment sites on the surface of the cell. If this type of tensegrity mechanism is used by cells, then externally-applied mechanical loads, such as those produced by gravitational forces, could affect complementary force interactions, change local thermodynamic parameters, and thereby alter cytoskeletal filament arrangements or assembly. Changes in cytoskeletal organization can, in turn, alter the distribution and hence, function of much of the cell's metabolic machinery. Thus, characterization of the fundamental mechanism by which mechanical forces regulate the cytoskeleton and control cell shape could provide insight into the mechanism of gravity sensation. Understanding how cell shape is controlled and how cells change their form and function in response to mechanical forces will likely also have important implications for a wide range of diseases that involve changes in mechanoregulation, including hypertension, atherosclerosis, musculoskeletal abnormalities, orthodontic remodeling, and cancer.

FY97 Publications, Presentations, and Other Accomplishments:

Ezzell, R.M., Goldmann, W.H., Wang, N., Parasharama, N., and Ingber, D.E. "Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton." *Exp. Cell Res.*, 231, 14-26 (1997).

Ingber, D.E. "Extracellular matrix: a solid-state regulator of cell form, function, and tissue development" in "Handbook of Cell Physiology." Edited by: Jamieson, J.D. and Hoffman, J.F. Oxford University Press: New York, pp 541-556 (1997).

Ingber, D.E. "Integrins, tensegrity, and mechanotransduction." *ASGSB Bull.*, 10, 49-55 (1997).

Ingber, D.E. "Tensegrity: The architectural basis of cellular mechanotransduction." *Annu. Rev. Physiol.*, 59, 575-599 (1997).

Ingber, D.E. First Meeting of External Advisory Board of the NASA Specialized Center on Research and Training (NSCORT) in Gravitational Biology, and Symposia, Houston, TX (April, 1997).

Ingber, D.E. "Architectonics of nature." Conference at Princeton University, Princeton, NJ (November, 1996).

Ingber, D.E. "Biological design principles that guide self-organization, emergence, and hierarchical assembly: From complexity to tensegrity." International Conference on Complex Systems, Nashua, NH (September, 1997).

- Ingber, D.E. "Biological structure and gene expression." Gordon Research Conference, Lucca, Italy (April, 1997).
- Ingber, D.E. "Cellular shapes and function." Meeting of the 6th International Congress on Cell Biology/36th American Society for Cell Biology Annual Meeting, San Francisco, CA (December, 1996).
- Ingber, D.E. "Cellular tensegrity and morphogenesis: From skeleton to cytoskeleton." British Biophysical Society Meeting, Manchester, United Kingdom (September, 1997).
- Ingber, D.E. "Dynamics networks in physics and biology." Third Les Houches Winter Workshop, Les Houches, France (March, 1997).
- Ingber, D.E. "Integrin signaling, cellular tensegrity, and control of angiogenesis." 17th International Congress of Biochemistry and Molecular Biology, San Francisco, CA (August, 1997).
- Ingber, D.E. "Integrin signaling, cellular tensegrity, and control of angiogenesis." University College, London, United Kingdom (September, 1997).
- Ingber, D.E. "Tensegrity architecture of cells." Third Cytonet Retreat, Purdue University, Breckenridge, CO (May, 1997).
- Ingber, D.E. "Tensegrity: The cellular basis of mechanotransduction." Visiting Professor for the Cell and Molecular Biology Program, Hershey, PA (April, 1997).
- Ingber, D.E. "The cellular basics of mechanotransduction." Marine Biological Laboratory, Woods Hole, MA (November, 1996).
- Maniotis, A., Bojanowski, K., and Ingber, D.E. "Mechanical continuity and reversible chromosome disassembly within intact genomes microscopically removed from living cells." *J. Cell. Biochem.*, 65, 114-130 (1997).
- Maniotis, A., Chen, C., and Ingber, D.E. "Demonstration of mechanical connections between integrins, cytoskeletal filaments and nucleoplasm that stabilize nuclear structure." *Proc. Natl. Acad. Sci. U.S.A.*, 94:849-854 (1997).

The Effect of Hypergravity on Bone Cell Cultures

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Funding:

UPN/Project Identification: 199-40-17-12

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$29,133

Task Description:

This application is directed to the NASA Space Physiology and Countermeasures Program concerning the physiological response of bone to hypergravity. We intend to examine the effect of hypergravity on cultures of bone cells in a series of ground-based experiments. In accord with the Program guidelines, the investigations are focused ultimately toward understanding mechanisms underlying bone loss during space flight with the long-range goal of preventing such loss. Studies will examine possible changes in bone cells and bone matrix in response to changes in gravity. The proposal will involve molecular biology, biochemistry, and structure and has two principal purposes, the first to extend our previous results characterizing a chicken bone culture system growing under normal gravity (1-G) and the second to compare these results with those obtained by loading (>1-G) the culture system in hypergravity generated centrifugally. These two objectives will test the hypothesis that loading evokes responses in the activity of bone cells (osteoblasts) resulting in changes in gene expression; cytoskeleton, integrin, and extracellular matrix composition and/or structure; and mineralization.

Specific aims will determine at 1-G the organization of non-collagenous proteins in the bone cultures, their means of secretion, and their possible interaction with mineral, and will characterize cytoskeletal elements and integrins as a function of time. These features, which have not been investigated, will be compared with those measured in the same cultures loaded at the Hypergravity Facility for Cell Culture (HyFaCC) at NASA/Ames Research Center. In addition, 1-G and >1-G comparisons will be made for parameters previously or currently studied at normal gravity and microgravity in other experiments. These will include cell growth measures and collagen and mineral formation and interaction. By our approaches, bone cell activity, function, and adaptation under normal and increasing gravitational load may be understood more completely. These data, and those correlated with our investigations of microgravity based on two recent NASA shuttle missions, would hopefully elaborate bone loss mechanism, leading to development of appropriate countermeasures to bone deterioration whether in space flight, conditions of bone disuse, or other situations.

Ascorbic acid is known to mediate collagen gene expression, synthesis, and post-translational processing in addition to extracellular matrix development in a variety of vertebrate connective tissues. The details of such ascorbic acid effects are incomplete, however. This laboratory has begun investigating the role of ascorbic acid in these aspects of a tendon culture model as a correlate to other current bone studies *in vitro*. Initial results show that tendon cells obtained from the legs of normal 17 day old embryonic chicks elaborate a collagenous

extracellular matrix in which type I collagen fibrils are assembled in a defined temporal and spatial sequence. The formation occurs through the accumulation of fibril arrays deposited between successive culture cell layers. The process is facilitated by the presence of ascorbic acid with an overall 2-3-fold increase in total cell layer thickness that appears to be associated with an increase in collagen fibril number and diameter. Ascorbate does not affect collagen mRNA levels but alters post-translational processing of the molecules. In this instance, cultures grown in the absence of ascorbic acid fail nearly totally to convert procollagen $\alpha 1(I)$ and $\alpha 2(I)$ chains to collagen while cultures supplemented with ascorbate convert nearly all the procollagen $\alpha 1$ and $\alpha 2$ species to the fully processed collagen chains. These data suggest that a major point of control for the accumulation of type I collagen within the cell layers of the tendon cultures is at the level of procollagen processing. Ascorbate would also appear to regulate the presence or activity of carboxy- and amino-propeptide peptidases. Additional studies are in progress to determine more precisely the effects of ascorbate in this tendon culture system maintained at normal gravity.

Besides following the ground-based studies described briefly above, the laboratory has been involved in discussions during FY97 with NASA/Ames Research Center directed toward planning and implementing experiments scheduled for January, 1998, and utilizing the Hypergravity Facility for Cell Culture. An on-site visit by the laboratory to NASA/Ames resulted in establishing contacts with key support personnel, familiarizing laboratory members with the activity and operation of the HyFaCC and Life Sciences Division, reviewing and defining experimental timelines and other requirements, coordinating supply and equipment needs, and identifying a database of previous cell culture micro- and hyper-gravity studies. Coordination of the experimental plans for acquiring information on the effects of hypergravity on the bone cell cultures developed by this laboratory is continuing.

The experiments proposed here to assess the responses of cultured bone cells subjected to hypergravity in the HyFaCC at NASA/Ames Research Center or to normal gravity are intended to define the means by which these cells function and adapt under changing environmental conditions. Data would be important with regard to understanding more completely a number of features of bone, including its principal role in the structural support of the body and its ability to increase in mass as a consequence of applied mechanical forces. Correlated with other information documenting the loss of bone mass in vertebrates during space flight (skeletal unloading) and results obtained by this laboratory following two recent NASA shuttle missions, in which gene expression of type I collagen developed by cultured bone cells appeared to be down-regulated, the data from both normal gravity and hypergravity experiments would describe more fully the adaptation of bone over a wide range of the gravitational spectrum. In so doing, these experiments may provide additional insight into determining a mechanism for bone mass accumulation or loss, the implications of which would be basic to new knowledge regarding bone behavior under a variety of conditions. These would include times of high mechanical or physical activity such as during exercise as well as intervals of bone disuse as in weightlessness, prolonged bed rest or other periods of inactivity, and during immobilization of limbs following bone repair and healing. The results would also hopefully provide concepts for counterbalance treatment and prevention of the bone mass decreases under the latter situations.

Are G Proteins Mechanosensors for Endothelial Cells?

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No Co-Is Assigned to this Task

Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1994

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

Limited investigation has been performed to determine the effect of gravity on signal transduction of mammalian cells, particularly vascular cells, despite the pronounced and well studied cardiovascular deconditioning that is known to occur during space flight. However, evidence is accumulating that physical forces can modulate endothelial cell (EC) and smooth muscle cell (SMC) phenotype and may influence the vascular response to injury. Although not identical to gravitation, how EC and SMC sense changes to mechanical perturbation such as cyclic strain may be pertinent to that which occurs in response to changes in gravity. The objective of the proposed studies is to examine the effect of mechanical signaling at the cellular level. Preliminary data demonstrate that acute cyclic strain of bovine aortic EC causes loss of immunoreactivity of the inhibitory G protein alpha subunits Gi(1,2), that correspond temporally to the activation of the adenylate cyclase signal transduction pathway. The specific hypothesis to be tested in these studies is that strain-induced loss of Gi(1,2) is caused by post-translational modification of this protein directed at the carboxyl terminus. The hypothesis is based on preliminary data that show strain-induced loss of Gi(1,2) is limited to antisera that recognize the carboxyl terminus of Gi(1,2). The carboxyl terminus of Gi(1,2) contains a CAAX motif that is a well recognized site of post-translational modifications such as prenylation, carboxymethylation, and ADP-ribosylation.

We have continued to make significant progress toward delineating the role of G proteins as mechanoreceptors. In particular, we have focused on the MAP kinase signaling pathway that is distal to activation of GTP-binding proteins. Recent studies demonstrate that cyclic strain stimulates proliferation of vascular smooth muscle cells and endothelial cells. However, the transduction events that mediate this response remain ill-defined. We tested the hypothesis that cyclic strain activates MAP kinase since it has been implicated in the regulation of cell cycle progression. Smooth muscle cells were synchronized to the quiescent state by a 48 hour exposure to serum deprivation (0.1% fetal bovine serum) followed by acute (0, 5, 10, 30, and 60 min) exposure to cyclic strain (10% average strain at 60 cycles/min). Following cyclic strain, Triton-solubilized extracts were subjected to Western blot analysis with monoclonal antibodies directed toward both erk-1 (44 kD) and erk-2 (42 kD). We found a preferential phosphorylation of erk-2 with a peak response observed at 10 min. These data demonstrate

cyclic strain activation of MAP and suggest a potential mechanism by which cyclic strain stimulates smooth muscle cell proliferation.

We have also examined the effect of cyclic strain on G-protein mediated gene regulation, in particular prostacyclin synthase expression in bovine aortic endothelial cells. Recent studies indicate that hemodynamic forces such as cyclic strain and shear stress can increase PGI₂ secretion by endothelial cells but the effect of these forces on prostacyclin synthase (PGIS) gene expression remains unclear and was the focus of this study. Bovine aortic endothelial cells (EC) were seeded onto type I collagen coated flexible membranes and grown to confluence. The membranes and attached EC were subjected to 10% average strain at 60 cpm (0.5 s deformation alternating with 0.5 s relaxation) for up to 5 days. PGIS gene expression was determined by Northern blot analysis and protein level by Western blot analysis. The effect of cyclic strain on the PGIS promoter was determined by the transfection of a 1kb human PGIS gene promoter construct coupled to a luciferase reporter gene into EC, followed by determination of luciferase activity. PGIS gene expression increased 1.7 fold in EC subjected to cyclic strain for 24 hours. Likewise, EC transfected with a pGL3B-PGIS(-1070/-10) construct showed an approximate 1.3 fold elevation in luciferase activity in EC subjected to cyclic strain for 2, 4, 8, and 12 hours. The weak stimulation of PGIS gene expression by cyclic strain was reflected in an inability to detect alterations in PGIS protein levels in EC subjected to cyclic strain for as long as 5 days. These data suggest that strain-induced stimulation of PGIS gene expression plays only a minor role in the ability of cyclic strain to stimulate PGI₂ release in EC. These findings, coupled with our earlier demonstration of a requisite addition of exogenous arachidonate in order to observe strain-induced PGI₂ release, implicates a mechanism that more likely involves strain-induced stimulation of PGIS activity.

The objective of these studies is to examine the effect of mechanical perturbation on endothelial and smooth muscle cell signaling at the cellular level. Studies to date support our original hypothesis that cyclic strain causes post-translational modification of the inhibitory G protein. Based on inhibitor studies, the nature of the post-translational modification appears to be ADP-ribosylation and not isoprenylation. This has been confirmed by *in vitro* experiments with pertussis toxin. These data suggest that G proteins act as mechanotransducers and thereby implicate a cellular mechanism by which endothelial cells may "sense" changes in gravity. We have also implicated other signaling molecules and genes as important players in mechanotransduction, including MAP kinase and prostacyclin synthase. Future studies with altered gravitational states, as well as flight studies, will be required to confirm our hypothesis.

FY97 Publications, Presentations, and Other Accomplishments:

Cohen, C.R., Mills, I., Du, W., and Sumpio, B.E. "Activation of adenylate cyclase, cAMP, PKA and CREB protein in bovine aortic endothelial cells exposed to cyclic strain." *Exp. Cell Res.*, 231, 184-189 (1997).

Ikeda, M., Takei, T., Mills, I., and Sumpio, B.E. "Cyclic strain stimulates mitogen-activated protein kinase activation in cultured bovine aortic endothelial cells." *FASEB J.*, 11, A458 (1997).

Mills, I., Cohen, C.R., Kamal, K., Li, G., Shin, T., Du, W. and Sumpio, B.E. "Role of PKC and PKA in strain-induced smooth muscle cell proliferation and alignment." *J. Cell. Physiol.*, 170, 228-234 (1997).

Mills, I., Ikeda, M., and Sumpio, B.E. "Map kinase activation by cyclic strain in vascular smooth muscle cells." *FASEB J.*, 11, A216 (1997).

Oluwole, B.O., Du, W., Mills, I., and Sumpio, B.E. "Differential gene regulation by mechanical forces." *Endothelium*, 5, 85-93 (1997).

Segurolo, R.J., Jr., Oluwole, B., Mills, I., Tanabe, T., Yokoyama, C., Kito, H., Nakajima, N., and Sumpio, B.E. "Cyclic strain is a weak inducer of prostacyclin synthase expression in bovine aortic endothelial cells." *J. Vasc. Res.* (In Press).

Takei, T., Han, O., Ikeda, M., Male, P., Mills, I., and Sumpio, B.E. "Cyclic strain stimulates isoform-specific PKC activation and translocation in cultured human keratinocytes." *J. Cell. Biochem.* (In Press).

Takei, T., Rivas-Gotz, C., Delling, C.A., Koo, J.T., Mills, I., McCarthy, T.L., Centrella, M. and Sumpio, B.E. "The effect of strain on human keratinocytes *in vitro*." *J. Cell Physiol.* (In Press).

Skeletal Collagen Turnover by the Osteoblast

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-40-47-02

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 100,450

Task Description:

We hypothesize that osteoblast-specific transcription factors regulate the expression of collagenase in normal, differentiating osteoblasts. The present study will test this hypothesis by i) determining the differentiation-specific element in the rat collagenase gene; ii) identifying the nuclear proteins which bind to this regulatory element; iii) purifying and identifying the transacting factors; and iv) cloning novel factors.

We have found that collagenase is expressed in normal differentiating rat osteoblasts. Expression of collagenase is greatest in the most differentiated cells at a time of greatest formation of mineralized nodules. We have determined that there is minimal transcription of the collagenase gene in proliferating osteoblasts, and that the gene becomes transcriptionally active when the cells are mineralized and differentiated. This was done by the method of nuclear run-on assays. Thus, the transcriptional rate is the determinant of changes in mRNA abundance. Now that we have this information, we have returned to the transfection experiments.

From other work with the rat osteosarcoma line, UMR 106-01, we have determined that the elements in the rat collagenase gene responsible for PTH regulating transcriptional activity are the AP-1 site, a basal element, and a runt domain (RD) binding site upstream of the AP-1 site, which appears to be an osteoblast-specific element in many bone genes. We have now done transfections of promoter constructs with each of these elements. In the proliferating cells, promoter activity is very low, correlating with the mRNA abundance and rate of transcription experiments for the endogenous gene. We have attempted to complete the difficult experiments with the mineralizing cells. After many different approaches to transfecting non-proliferating, mineralized cells, we found that calcium phosphate co-precipitation was the best, but still only worked occasionally with mineralizing cells. The preliminary data suggest that the two elements identified to be the PTH-responsive elements are also responsible for regulation of differentiated expression of the collagenase gene in normal osteoblasts.

We have started an alternative approach to determine if the elements operating in PTH regulation of collagenase in the transformed osteoblasts are the same in differentiating normal osteoblasts. To do this, we are co-transfecting the proteins shown to bind to these elements in the UMR cells, the AP-1 members, Fos and Jun, as well as the CBF members, CBFA1 and CBFβ. This will be done in two approaches, either examining collagenase mRNA expression, or collagenase promoter expression in proliferating osteoblasts. The hypothesis is that the two groups of transcription factors may switch on expression of collagenase in differentiating

mineralizing osteoblasts and if we provide these to the proliferating cells, the same may occur. We have preliminary data that the first approach will work.

Concurrently, we are also obtaining information as to what proteins bind to these two elements in the normal differentiating osteoblasts. Two approaches are being taken to do this: The first is the gel mobility shift assay, which determines whether proteins will bind to a specific sequence of DNA. Using the RD binding site sequence, we have found that there is a novel shifted band in the mineralizing cells compared with the proliferating cells. We also know that the proteins binding to this element are members of the acute myelogenous leukemia (AML) family (also called CBF, core binding factors) of human transcription factors. We are undertaking experiments to also assess the expression of members of this family by Western and Northern blots. We are also examining expression of the AP-1 members by the same means.

The osteopenia due to weightlessness appears to be manifested by a change in the functions of the osteoblast. This cell has been shown to have stretch receptors and may be the gravity-responsive cell in bone which possesses the putative "mechanostat." The latter is thought to sense changes in load and cause the adjustment of bone mass. Under conditions of decreased load (e.g., microgravity), this may be affected by a reversal in maturation of the osteoblast. The present proposal will determine the mechanisms involved in the appearance of expression of collagenase by normal differentiating osteoblasts. These studies should add to our knowledge of the regulatory pathways influencing skeletal mass and calcium homeostasis and will lead to similarly focused experiments in space. The work will aid in our understanding of loss of bone in osteoporosis and osteopenia due to a decrease in loadbearing or immobilization.

FY97 Publications, Presentations, and Other Accomplishments:

Koe, R., Clohisy, J.C., Tyson, D.R., Pulumati, M.R., Cook, T.F., and Partridge, N.C. "Parathyroid hormone versus phorbol ester stimulation of activator protein-1 gene family members in rat osteoblastic cells." *Calcif. Tiss. Int.*, 61, 52-58 (1997).

Partridge, N.C. and Winchester, S.K. "Osteoblast proteinases" in "Principles of Bone Biology." Edited by: Bilizekian, J.P., Raisz, L.G., and Rodan, G.A. Academic Press, San Diego, pp 207-216 (1996).

Pearman, A.T., Chou, W.-Y., Bergman, K.D., Pulumati, M., and Partridge, N.C. "Parathyroid hormone induces c-fos promoter activity in osteoblastic cells through phosphorylated CREB binding to the major CRE." *J. Biol. Chem.*, 271, 25715-25721 (1996).

Functional Correlates of Genetic Expression Induced by Hypergravity

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Funding:

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Expiration: 1999

Students Funded Under Research: 1

Post-Doctoral Associates: 1

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Task Description:

This project addresses questions related to the Space Biology program emphasis to examine the cellular mechanism of adaptation to acute variation in gravity by relating genomic activation to altered physiological function. A set of ground-based experiments are proposed to assess the effects of short-duration exposure to hypergravity or expression of immediate-early-gene related transcription factors in brainstem neurons of gerbils. Those molecular responses will be correlated with measurement of vestibulo-ocular functions. Genomic expression will be suppressed by the regional infusion of acute-sense oligonucleotides to determine whether molecular changes in specific groups of neurons are essential for physiological effects of hypergravity. Additional functional assessments will be made by measurement of the responses of brainstem neurons to natural vestibular stimulation in the form of linear and angular head acceleration prior to and following exposure to specific vectors of 2 X g linear force.

The following is a summary of progress made during fiscal year 1997 for the subject project. Among the Specific Aims of this project was an evaluation of the efficacy of antisense oligonucleotides as a tool for investigating the molecular mechanisms regulating adaptation capabilities of the vestibular system. In our previous investigations, we demonstrated that the immediate early gene *c-Fos* induces a transcription factor (the protein *Fos*) in the nuclei of selected neurons in specific nuclei within the vestibular related complex and in other nuclear areas of the brainstem and cerebellum following application of hypergravity (centripetal acceleration). Novel stimulation to the vestibular system is required for expression of that gene. We have initiated our studies during fiscal year 1997 to investigate the efficacy of that technique by utilizing lesions of the vestibular end organ as a method to initiate a process of vestibular plasticity known as vestibular compensation. We assessed activation of the gene by immunohistochemical techniques for labeling the protein product *Fos* expressed in the nuclei of the affected neurons. In order to block *Fos* expression, we obtained highly characterized antisense oligonucleotides directed toward the gene regulating *Fos*. This agent was applied, to specific nuclear areas containing reactive cells, by microinjections of solutions of the oligonucleotide applied through stereotaxically implanted cannulae. First, we demonstrated that the antisense agent produced a highly localized blockage of *Fos* expression at the injection site. In our studies to date, we have demonstrated that vestibular compensation in a gerbil animal model, during the first hour of compensation following unilateral ablation of the vestibular sensory organs, can be markedly altered by infusion of anti-*Fos* phosphorothioate oligonucleotide into vestibular related regions such as the medial vestibular nucleus, the nucleus prepositus, or the inferior olivary nucleus. The specific behavioral effects differed according to the specific area receiving the

injection and the amount of reduction of Fos expression produced by those infusions. We have determined that the volume of injection, the concentration of the oligonucleotide, the incubation time before surgery, the test environment, and intersubject behavioral variability all are significant sources of variance.

Unilateral ablation of vestibular sensory organs induces postural and locomotor asymmetries. Among those is a marked tendency for animals to circle toward the side of the labyrinthine lesion and to fall toward that side when trying to stand on their hind quarters. Ipsilateral circling is significantly decreased in animals following anti-Fos oligonucleotide injection into the inferior olive. Anti-Fos injections into the medial vestibular or prepositus regions both reduce ipsilateral circling and extend the duration of time over which that behavior is exhibited. Circling in the direction opposite to the lesion side is rarely observed in control animals. Anti-Fos oligonucleotide injection into the medial vestibular and prepositus areas increased that behavior, which counters the normal ipsiversive dominance in locomotor behavior following such lesions. These experiments pave the way for subsequent studies that will target other substances that are expressed following the activation of such specific transcription factors like Fos in specific nuclear areas such as the ones involved in our studies to date. Concurrently with behavioral measures, we have also begun to evaluate the effects of blockage of Fos expression on such functions as vestibulo-ocular responses both in labyrinth intact and hemilabyrinthectomized animals. This work was presented at the annual meeting of the Association for Research in Otolaryngology. A second abstract was presented at the 1997 meeting of the Society for Neuroscience. A manuscript has been submitted to the *Journal of Brain Research*.

The third objective of this project was to assess vestibular responses of brainstem neurons and to determine how those responses might be affected by exposure of the animal to hypergravity. This year, our focus has been on studies of neurons in the prepositus nucleus, which is a vestibular related area that contains a large percentage of neurons that exhibit Fos expression following application of hypergravity stimuli generated by centripetal acceleration. In previous studies in this laboratory, we reported that neurons in the medial vestibular nuclei of decerebrated rats in large measure receive vestibular inputs from afferent fibers related to both the otolith organs and the semicircular canals. Those neurons were found to respond to multiple vectors of linear head acceleration. Their response amplitudes and response phases were found to be a function of vector polar angle, as referred to head coordinates. Thus, unlike the primary vestibular afferents, second and higher order vestibular nuclei neurons appear to encode head acceleration to reflect both spatial and temporal convergence (STC). We have examined the response properties of both decerebrated and alert preparations for neurons in the prepositus nucleus. This set of studies was required in order to perform subsequent experiments to examine the correlations between neurophysiological data and the occurrences of Fos expression. Although, the phenomenon STC was present in neurons from both decerebrated and alert preparations, several statistically significant differences were found. Those included increased background discharge rates and larger response amplitudes in the prepositus neurons of alert preparations. In addition, a large percentage of those neurons exhibit signals related to ocular position or ocular velocity. They also are sensitive to visual inputs, generally demonstrating increased response gains when vestibular stimulation is applied in the light; similarly, vestibulo-ocular responses, as noted by numerous other investigators, also increased with combined dynamic visual and vestibular stimulation. Data have been obtained thus far from over 200 neurons using linear and angular acceleration test protocols. These control studies are near completion thus providing the database necessary for studies in progress under conditions designed to induce vestibular adaptation and activation of immediate early gene responses.

In order to develop a relationship between human and animal models of vestibular adaptation, we are coordinating our work with studies directed by Dr. W. Paloski. The liaison for this work is Dr. Galen Kaufman, a former NIH fellow in this laboratory, and presently an NRC fellow at the JSC. The following is a brief description of that work.

Dr. Kaufman, in collaboration with Dr. Paloski at NASA JSC, is beginning a small-arm centrifuge (SAC) study which will evaluate gravitational reference changes in subjects following short-term hypergravity. The stimulus will consist of 60 to 90 minutes of constant 1.4 G centripetal acceleration (horizontal rotation) in the Gy (frontal) plane in the dark with a horizontal head movement task. Postural (sway and center of gravity) and eye reflex (VOR, OVAR, and post-rotatory tilts) dependent measures will be evaluated in an attempt to quantify

the time course and vector direction of perceived shifts in Earth vertical. The research will address the theory that altered gravitational environments cause a central reorganization from a gravity to a head-based frame of reference. Subsequent animal experiments using the same stimulus parameters will explore possible neural substrates of these phenomenon. Thus, we hope to establish a functional linkage among molecular processes, neurophysiological responses, and perceptual adaptation to gravito-inertial changes. Our animal studies of vestibular compensation may yield insights into molecular mechanisms that may be manipulated to improve behavioral recovery in patients suffering vestibular dysfunction.

Hyper-G Studies of Vestibular Maculas Neural Plasticity

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Funding:

UPN/Project Identification: 199-40-12-01
Initial Funding Date: 1995
Students Funded Under Research: 0
FY 1997 Funding: \$ 131,000

Solicitation: 93-OLMSA-07
Expiration: 1998
Post-Doctoral Associates: 0

Responsible NASA Center: Ames Research Center

Task Description:

The long-term goal of this combined morphological/electrophysiological investigation is to increase understanding of vestibular macular adaptation to altered gravity. The research builds upon and extends previous morphological findings of synaptic plasticity in maculae of rats exposed to altered gravity. A goal was to complete analysis of flight data already in hand while the base work for electrophysiological studies of vestibular nerve fibers progressed to implementation. The new investigation would focus on the correlation between synapse structure and distribution and the electrophysiological properties of primary afferents adapted to hypergravity and during readaptation to Earth's 1-G. For the electrophysiological research, new probes that included chips for simultaneous collection of data from as many as 8-10 different afferents were to be employed. The intent of the three year proposal, whose period has now ended, was as follows: During the first year, morphological studies of maculae of rats centrifuged at 2-G for 14 days and for four days were to be conducted to compare findings with SLS-2 results and to examine adaptive effects at this early stage. This tissue was to be embedded and a pilot study was to be carried out. The results of the first year anatomical effort indicated that synapses of type II hair cells declined in hypergravity, an effect opposite to that observed in microgravity on SLS-2. This indicated that a correlated study of centrifuged rats would be worthwhile. In the meantime, analytical studies of SLS-2 data would continue as a basis for analysis of the data from centrifuged animals. Synapse type and distribution were to be characterized using 3-D software developed in the Biocomputation Center, while statistical analysis of variance would be carried out using SuperANOVA software. For the electrophysiological study, a ground-based study was also to be completed in year one and correlated anatomical and physiological studies would begin following that. Rats were to be centrifuged for four days at 2-G initially, since the results would be relevant to planning space research on early adaptive changes. Rats chronically implanted with electrodes were to be tested on a linear sled in populations exposed to hyper-G and during readaptation to 1-G, with electrophysiological events recorded. Spontaneous and stimulated firing rates were to be analyzed for rate, gain, coefficient of variation, and functional polarization vector. New understanding of macular dynamics, plasticity, and behavioral responses should emerge from such a correlated anatomical and physiological investigation. The information generated would be useful in planning future experiments on neural plasticity in altered gravity environments.

Work accomplished successfully on this task during 1997 was largely anatomical in nature. That is, ultrastructural studies of morphological changes in rat utricular maculae resulting from exposure to microgravity for 14 days were completed. Data on synapses from this SLS-2 experiment were subjected to analysis of variance (ANOVA) and also to multivariate analysis (MANOVA) using features of the SuperANOVA software. A report has now been prepared and is ready for submission for publication. Briefly, changes in type II hair cells far exceed those in type I cells (ANOVA). Also, day plus microgravity both affected synapses in type II cells but only microgravity affected type I cells (MANOVA). The results of this effort made us ready to begin the analysis of utricular maculae from rats exposed to hypergravity. We have collected data from three samples so far, and are stressing findings in days 1 and 4 of hypergravity. Control and centrifuge data are studied without the analyst's knowledge of which is being used. Earlier electrophysiological research under this grant had shown that Scarpa's ganglion, from which the data were to be collected, could be successfully penetrated by sample multichannel electrodes supplied by the researcher at the University of Michigan who oversees their manufacture. However, several bad runs of probes impeded progress during the year and the electrophysiological research planned could not be conducted. This research will be picked up by a National Research Council Associate working with Drs. Tomko and Ross, using conventional probes until the supply of multichannel probes using chip technology is dependable.

The SLS-2 and hypergravity research has already resulted in new understanding of the fundamental circuitry of the vestibular maculae (gravity sensors). That is, there are direct and local microcircuits, involving type I and type II hair cells respectively. It is the type II cells of the local microcircuits that exhibit most change in microgravity (synapses increase) or hypergravity (synapses decline). It is of great interest that only type II cells are affected in hypergravity at the single time studied (14 days, 2-G). In microgravity, both types of cells are affected but type II cells show the greater changes. The research is indicating that gravity is a continuum as a stimulus to type II hair cells, which are possibly the prime sensors of gravitational force. Beyond this new knowledge of morphology and plasticity differences in the hair cells, the research seeks to answer another fundamental question. This is whether plasticity in synaptic kind, number, and distribution in altered gravity results in initial differences in electrophysiological responses that then subside as the system is returned to a more typical output. That is, are plastic changes in this endorgan simply an attempt to achieve normalcy in output by a challenged system? The findings are relevant not only to increasing understanding of plasticity resulting from exposure to altered gravity, but to understanding plasticity in gravity receptors resulting from other causes. The research will have broad applications in fundamental science as well as targeted ones. Results will prove useful to clinicians studying various diseases of the vestibular system, to neuroscientists engaged in studies of neuronal plasticity at other sites, and to researchers studying the causality of Space Adaptation Syndrome. Additionally, work with newly developed multichannel electrodes, when the technology is mature, will greatly improve our knowledge of the simultaneous activity in several different nerve fibers. Each of the nerve fibers responding to the same input has slightly different neuronal connectivities. Coding of sensory information requires transfer centrally by assemblies of neurons, but the simultaneous responses of an assembly of nerve fibers is unknown for gravity sensors. Little is known about responses of assemblies of neurons elsewhere. Thus, the use of the electrodes described here is a cutting edge technology that will be applied more generally by other electrophysiologists in the future. However, we must wait until the supply of such electrodes is sufficient to meet the needs of investigators who must implant several animals in order to obtain statistically significant data.

FY97 Publications, Presentations, and Other Accomplishments:

Ross, M.D. "Morphological evidence for local microcircuits in vestibular maculae." *J. Comp. Neurol.*, 379, 333-346 (1997).

Ross, M.D. "Synaptic changes in rat maculae in space and medical imaging: The link." *Otolaryngol. - Head & Neck Surg.* (In Press).

Ross, M.D. "3-D displays and virtual systems." (Invited Speaker) *Advanced Technologies for Gravitational Biology Workshop*. Ames Research Center, Moffett Field, CA (January 14, 1997).

Ross, M.D. "Adaptive responses of rat vestibular macular hair cells to microgravity isolation and other stress." Association for Research in Otolaryngology, St. Petersburg, FL (February 1 - 6, 1997).

Ross, M.D. "Neurovestibular system and virtual surgery." NASA Ames Research Center Open House, Moffett Field, CA (September 20, 1997).

Ross, M.D. "Studies of neuroplasticity in space." International Workshop on Neuroscience in Space, Paris, France (April 22 - 24, 1997).

Ross, M.D. "The role of space in the exploration of the mammalian vestibular system." (Invited Speaker) 12th Man in Space Symposium, Washington, DC (June 8 - 13, 1997).

Transgenic Markers of Bone Cell Lineage Progression

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Funding:

UPN/Project Identification: 199-40-27-14
Initial Funding Date: 1996
Students Funded Under Research: 0
FY 1997 Funding: \$68,300

Solicitation: 95-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 2

Task Description:

Bones sense the load they are required to bear and alter their architecture to compensate, either increasing strength when mechanically loaded or losing strength when unloaded. One of the key events in the remodeling process is the appearance bone-forming osteoblasts at the sites where new bone needs to be made. These cells are thought to arise from a pluripotential stem cell which proliferates and differentiates through a number of steps into a mature bone-forming osteoblast. We have developed a family of collagen-promoter transgenes which appear to be activated at different stages in the bone cell lineage. When expressed in transgenic mice, these can serve as convenient and powerful markers to assess the process of recruitment in intact mice exposed to physiological stimuli of skeletal loading or unloading. This research will develop a mouse model that will be useful to follow the cellular response to gravitational changes on the skeleton. The activation of these transgenes will be assessed by standard and immunohistomorphology of bone (by Dr. Yeh) and in marrow stromal cells (Dr. Rowe) derived from mice subjected to a mechanical loading (treadmill) or unloading (swimming). If these transgenes are shown to accurately reflect the cellular activities, then the model could be used to evaluate strategies for preventing bone loss during prolonged space flight by measuring transgene signals that are secreted into the blood so that the temporal response can be monitored without having to sacrifice the animal.

Our first year has been spent trying to develop the conditions that will induce an osteoblastic response in long bones to mechanical loading and unloading in an outbred CD-1 sexually mature mouse. The initial experiments examined the response to daily treadmill exercise (up to 2 hrs/day) for three continuous weeks. No effect was observed in two attempts using increasing amount of exercise. We reasoned that the control mice were getting equivalent exercise, but it occurred a night when spontaneous burrowing activity occurs. Attempts to discourage this activity by placing cotton bedding in the cage failed. Next we compared mice that got similar physical activity but in a different form: treadmill versus swimming. The hope was that the two groups would be equally exhausted from the exercise and would limit their nocturnal burrowing. Any difference in bone formation would be related to the type of experimental exercise they experience. The result of this experiment was negative. No molecular or histomorphological difference between the two groups or an unexercised control was observed.

Based on our negative data plus preliminary positive data for other investigators using the C57/B6 mouse which is known to have inherently small bones, we have concluded that an outbred mouse has maximally formed bones which are not forced to increase their strength when subjected to mechanical loading, nor which resorbs bone in

response to the few hours of unloading that occurred with swimming. To overcome this problem, we are designing experiments to be performed of C57/B6 mice and the heterozygous OI mouse (OIM/+) both of which do not have an obvious bone problem, but do have subclinical evidence of osteopenia or bone weakness.

The other area of activity is the development of new mice that will be useful in evaluating the osteoblastic response to mechanical loading in an intact animal. The first series of COL1A1 driven GFP transgenic mice failed to give a signal either in intact tissue or cultured marrow stromal fibroblast derived bone nodules. A new generation of GFP marker genes in which the encoded protein assembles efficiently at 37 degrees have recently become available (GFP emerald and topaz). In our hands, these autofluorescent proteins give a extremely strong signal relative to other versions in transfected cell lines. Transgenic mice with this version of GFP are currently being made.

We are exploring the possibility of developing new marker genes for the osteoblast lineage prior to expression of the COL1A1 bone element. One candidate is the ALCAM (CD166) surface marker described by Dr. S. Bruder that is expressed in the earliest proliferating cells that are derived from marrow stromal cells. It probably represents a progenitor that is common to the entire bone/cartilage/adipocyte lineage. A second surface marker developed by Dr. M. Horowicz probably appears after the cells commit to the bone lineage. We are developing experiments to evaluate these two antigens and genes as markers of the lineage and determine if they will be helpful in assessing the osteoprogenitor response to mechanical stimulation.

A model for a bone loading and unloading that can be easily standardized and applicable to transgenic mice opens a way to study the molecular and cellular controls of gravity on intact bone. Models that use cultured cells lack this essential ingredient. Other models in intact rats that use tail lift, limb restraints, or partial neurotomy are far from physiological. Interpretation of experimental results are always clouded by secondary effects that might not arise in a more physiologically relevant model. We chose treadmill exercise of a sexually mature male mouse as the most relevant physiological model for space flight, but have had difficulty showing an exercise effect relative to caged non-exercised mice.

To overcome the problem with the outbred mouse model, we will be switching to mice with a defined abnormality of bone (OIM/+) or one in which a defective bone part of the inbred strain (C57/B6). If this proves to show that inherently weaker bones are required to show a osteoblastic response to mechanical loading, it will prove to be a valuable model to assess the effect of subtle mutation of genes that are expressed in bone. In particular would a knockout mouse for osteocalcin, BSP, osteopontin, or biglycan reveal an abnormality in its response to mechanical loading? Mice bearing these genetic deficiencies may have subtle abnormal histologic features, but their skeletons appear to be of sufficient strength that fractures do not occur. However, if the skeleton was stressed, would a defect be uncovered? Could such subtle defects give insight into biological variation of skeletal strength in humans which are aggravated in the postmenopausal years or during prolonged weightlessness?

New autofluorescent transgenes are being incorporated into the cell specific promoters so that bone cell lineage progression can be assessed in the transgenic mice with greater ease and discrimination. We hope that the transgenic mice that we have produced and a method to exercise or float mice can become a standardized and reproducible model so that comparison of different aspects of mechanical loading of bone can be interpreted from one laboratory to another.

FY97 Publications, Presentations, and Other Accomplishments:

Breault, D.T., Lichtler, A.C., and Rowe, D.W. "Col1A1 transgene expression in stably transfected osteoblastic cells: Relative contribution of the first intron, 3' flanking sequences and sequences derived from the body of the human COL1A1 minigene." *J. Biol. Chem.* (In Press).

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Dodig, M., Kronenberg, M.S., Bedalov, A., Kream, B.E., Gronowicz, G., Clark, S.H., Mack, K., Rowe, D.W., and Lichtler, A.C. "Identification of a homeodomain binding site required for high level expression of a COL1A1 promoter-CAT construct in differentiated osteoblasts of transgenic mice." *J. Biol. Chem.*, 271, 16422-16429 (1996).

Krebsbach, P.H., Kuznetsov, S.A., Satomura, K., Emmons, R.V.B., Rowe, D.A., and Gehron Robey, P. "Bone formation *in vivo*: Comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts." *Transplantation*, 63, 1059-1069 (1997).

Rowe, D.W. and Shapiro, J.R. "Diseases associated with abnormalities of structural proteins" in "Textbook of Rheumatology." Edited by: Kelly, W.M., Harris, E.O., Ruddy S., and Sledge, C.B., 5th edition. W.B. Saunders (In Press).

Rowe, D.W. and Shapiro, J.R. "Osteogenesis imperfecta" in "Metabolic Bone Disease." Edited by: Avioli, L. and Krane, S., 3rd edition. W.B. Saunders (In Press).

Shapiro, J., Primorac, D., and Rowe, D. "Current concepts of osteogenesis imperfecta" in "Principles of Bone Biology." Edited by: Bilezikian, J., Raisz, L., and Rodan, G. Academic Press, pp 889-902 (1996).

Integrated Analysis of Columella Cell Structure and Function

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Funding:

UPN/Project Identification: 199-40-57-49

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2001

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$128,202

Task Description:

The cellular and molecular mechanisms by which the gravity-sensing cells (statocytes) of higher plants transduce the weight or motion of amyloplasts into a biochemical and cell metabolic response is not well understood. Research to date suggests that amyloplast weight transduces the gravity signal through actin-based microfilaments or microfilament networks to the plasma membrane, where ion fluxes induce a signaling cascade that leads to the growth response. The observation of polar calcium gradients in the cell walls of gravistimulated cells suggests, furthermore, that the microfilament signal transduction pathway could induce a callose-dependent closure of plasmodesmal communication among subsets of cells, thereby redirecting the flow of growth regulatory molecules. The general goal of this study is to test experimentally critical predictions of the hypotheses described above. The novelty of this research resides in the use of new state-of-the-art methodologies (cryofixation, freeze-etch electron microscopy, computer axial tomography, optical trapping, microinjection of tracer molecules, and new mathematical modeling approaches) together with a new technique for isolating columella cells from root caps. The specific objectives include: 1) to biochemically characterize the cytoskeletal proteins in the cytosol of columella cells; 2) to determine if microfilaments tether amyloplasts to the plasma membrane as well as to each other; 3) to determine if pulling on one amyloplast will drag others along and measuring the force needed to move individual amyloplasts; 4) to relate cell wall calcium changes to possible callose deposition; 5) to follow the effects of gravity field changes on the flow of fluorescent tracer molecules between root cap cells; and 6) to use inferential control theory to develop new mathematical models of the gravity sensory response. Taken together, the new findings should enable us to formulate more sophisticated and novel types of experiments on future Space Shuttle and International Space Station missions.

This grant started on March 1, 1997. Most of the work to date has involved hiring and training research personnel, and engineering graduate student, Jim Clawson (June 1 start), a postdoctoral fellow, Dr. Hui-qiong Zheng (October 1 start), trained in cell and molecular biological techniques, and an undergraduate independent study student, Claire Langford (September 1 start). Jim Clawson has now designed the necessary hardware for the laser tweezer experiments and has recently ordered the parts. Dr. Zheng and Claire Langford are currently learning advanced electron microscopical techniques.

The research supported by this grant is based on novel cell biological insights gained from studies of plants grown on two Space Shuttle experiments. These studies suggested that the gravisensing apparatus of plants includes cytoskeletal elements that link the statoliths of each cell together into a functional unit. Theoretical

considerations suggest that this coupling increases the sensitivity of the gravisensing apparatus. The principle goal is to experimentally verify the existence of these cytoskeletal linkers and then characterize their properties. In the long term, this information could lead to new methods for controlling plant growth.

Mechanosensitive Ion Channels in Bacteria

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

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Solicitation: 95-OLMSA-01

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$165,432

Task Description:

The vast majority of organisms respond to touch, vibration, gravity, and changes in osmotic pressure. However, the molecular mechanisms of these phenomena remain largely unknown. Recently, we have succeeded in biochemical identification and cloning of the first mechanosensitive channel, isolated from *E. coli* cell envelope (MscL). The sequence predicts a unique 15-kD protein with highly hydrophobic core and a hydrophilic C-terminus. This project combines structural and functional studies of this first identified mechano-sensitive protein using advanced molecular and biophysical approaches. We will study the topology of protein folding and the stoichiometry of functional channel assembly with biochemical and molecular techniques. Functional roles for certain protein domains will be determined by site-directed mutations followed by functional patch-clamp assays and computer analysis of single-channel recordings. We will try to understand the nature of interactions within the channel conferring a mechanical compliance to the protein complex. Validation of the elemental principles of mechanosensitive protein functioning in eucaryotic MS channels, found in this relatively simple system, will be the final goal of this project.

During FY97, this research was focused on the character of MscL assembly and the mechanism of its gating. We have been able to obtain a more direct evidence that the functional MscL complex is a hexamer of 15 kD subunits, and to answer the question whether its structure is stable or represents a dynamic equilibrium with monomers. More specifically, we tried to determine whether membrane tension drives the MscL assembly from monomers, or whether stretch-induced gating transitions occur in pre-assembled channel complexes. Using biochemical techniques I found that upon mild extraction, the MscL protein is present as a population of uniform particles much heavier than the predicted monomer. The results of analytical centrifugation of tag-purified MscL are consistent with a hexameric structure for the complex, but also reveal a strong tendency to spontaneous precipitation. No monomeric form of MscL was detected. Generation, functional analysis, and *in situ* cross-linking of double and triple subunit tandems expressed as a single polypeptide confirm that the hexamer is the preferential mode of MscL assembly. Time-resolved recording and analysis of single-MscL currents appear to be another useful approach in studies of the dynamics of subunits and their interactions within the complex. Typical MscL traces show abrupt channel openings and a preferential occupation of either closed or fully open state interrupted with multiple short-lived subconducting states indicating some probability of non-concerted transitions in subunits within the complex. Peculiarly, closed-to-open and reverse transitions often proceed via similar substates. The estimated characteristic time of these transitions is 0.1-0.2 ms, and is independent of the protein concentration in the membrane, thus practically excluding the involvement of protein

diffusion in the entire mechanism of MscL gating. A primary analysis of records suggests that MscL is a cooperative system with a high degree of intersubunit coupling, although more experiments are required to draw a quantitative description of MscL. We conclude that MscL gating is a tension-driven conformational transition in pre-assembled channel complexes. More experiments are required to draw a quantitative description of MscL.

This research leads to understanding of very basic mechanisms of force detection by specific biological macromolecules in living organisms. It relates to primary mechanisms of mechanosensation, which encompass a wide range of phenomena from simple osmoregulation in bacteria to complex phenomena such as gravitropism in plants and balance and hearing in humans. MscL represents the first identified and characterized mechanosensory system in bacteria. It provides a highly useful system for a wide range of molecular, biochemical, and biophysical experiments and should be considered as a model. It is difficult to foresee any specific biomedical application of these basic studies within two or three years.

FY97 Publications, Presentations, and Other Accomplishments:

Blount, P., Sukharev, S.I., Moe, P., and Kung, C. "Mechanosensitive channels of *E. coli*: A genetic and molecular dissection." *Biol. Bull.*, 192, 126-127 (1997).

Ermakov, Y.A., Averbakh, A.Z., Shapovalov, V.L., and Sukharev, S.I. "Quantitative analysis of Gd³⁺ binding to liposomes, planar bilayers and lipid monolayers." *Biophys. J.*, 72(2), A71 (1997).

Sigurdson, W.J., Sukharev, S.I., Kung, C., and Sachs, F. "Energetic parameters for gating of the *E. coli* large conductance mechanosensitive channel (MscL)." (Abstract) *Biophys. J.*, 72(2), A267 (1997).

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Gravitational Effects on Signal Transduction

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No Co-Is Assigned to this Task

Funding:

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Initial Funding Date: 1995
Students Funded Under Research: 0
FY 1997 Funding: \$ 130,508

Solicitation: 95-OLMSA-01
Expiration: 1999
Post-Doctoral Associates: 1

Task Description:

An understanding of the mechanisms by which individual cells perceive gravity and how these cells transduce and respond to gravitational stimuli is critical for the development of long-term manned space flight experiments. We now propose to use a well-characterized model erythroid cell system and to investigate gravitational perturbations of its erythropoietin (Epo) signaling pathway and gene regulation. Cells will be grown at 1-G and in simulated microgravity in the NASA Rotating Wall Vessel bioreactor (RWV). Cell growth and differentiation, the Epo-receptor, the protein kinase C pathway to the c-myc gene, and the protein phosphatase pathway to the c-myb gene will be studied and evaluated as reporters of gravitational stimuli. The results of these experiments will have impact on the problems of 1) gravitational sensing by individual cells, and 2) the anemia of space flight. This ground-based study also will serve as a Space Station Development Study in gravitational effects on intracellular signal transduction.

We have carried out studies using the Rauscher murine erythroleukemia cell line, an *in vitro* model system that closely resembles normal erythropoiesis. We initiated cultures of log phase Rauscher cells in tissue culture flasks at 1-G, and simultaneously in the simulated microgravity environment of the NASA RWV. Whereas cells inoculated into tissue culture flasks continued their log phase growth pattern, cells in the RWV reproducibly underwent an apparent growth arrest that lasted approximately 12 hours. No difference in cell death or apoptosis was detected. Interestingly, after 24 hours, the growth rate in the RWV was essentially identical to that at 1-G, suggesting that the cells are able to accommodate that the new environment, as least as far as proliferation is concerned. Importantly, cells growing in the RWV exhibited a suboptimal response to erythropoietin. Addition of the growth factor to cells grown in flasks resulted in brisk hemoglobinization, reaching up to 25% of the cells within 48-72 hours. In marked contrast, cells in the RWV achieved only 5-10% hemoglobin positive cells in the same time period. Similar results were obtained in several repeat experiments. The data support our hypothesis that one or more pathways of erythropoietin's signal transduction cascade are impaired under conditions of simulated microgravity. This is consistent a role for impaired signal transduction in the anemia of space flight.

The results obtained thus far are consistent with the physiology of the anemia of space flight and support our proposed further studies of erythropoietin's signal transduction pathways. The anemia of space flight is a complex syndrome characterized in part by a blunted response to erythropoietin resulting in reduced red blood cell

production. This problem must be addressed. In addition, on Earth we see a similar blunted response in a variety of disease states, including the anemia found in cancer patients. It is possible that these two diverse conditions share some biochemical/molecular defects and that these defects in intracellular signaling can be modeled in the NASA RWV. Our further studies will dissect the signaling pathways triggered by erythropoietin and will identify those that are affected by microgravity. The results of this experimental approach could lead to new therapies for numerous anemic states, both on Earth and in space.

FY97 Publications, Presentations, and Other Accomplishments:

Sytkowski, A.J. "Erythroid cell growth and erythropoietin-induced differentiation are down-regulated in simulated microgravity." Presentation at the NASA Biotechnology Cell Science Program Investigators Working Group (IWG) Meeting. Houston, TX (February 27 - March 1, 1997).

Growth Factors and Tension-Induced Skeletal Muscle Growth

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Funding:

UPN/Project Identification: 199-40-47-03

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 6

Post-Doctoral Associates: 1

FY 1997 Funding: \$215,548

Task Description:

Three-dimensional mammalian skeletal muscle organs (organoids or bioartificial muscles, BAMs) will be generated in tissue culture with computer-controlled mechanical cell stimulators. They will be used to study tension/gravity-related skeletal muscle growth at the cellular and molecular level. The synergistic interaction of defined growth factors and mechanical forces in regulating muscle size will be analyzed in detail. Methods will be developed to grow the organoids in modified bioreactor cartridges of the Shuttle's Space Tissue Loss Module. Finally, the feasibility of "myofiber" gene therapy for the treatment of skeletal muscle wasting will be examined by studying the growth of BAMs implanted into syngeneic hosts, and then studying the reversal of hindlimb suspension-induced atrophy in animals implanted with genetically modified BAMs secreting recombinant growth hormone. The long-term goals of this project are to establish mammalian muscle BAMs as an appropriate system to study exercise attenuation of tension/microgravity-induced skeletal muscle atrophy in tissue culture, *in vivo*, and in space. Results from these studies will address one of the critical questions in space biology today — what chemical signals interact with tension/gravity to regulate tissue size? Although the current studies will cover only ground-based studies, we anticipate subsequent proposals to utilize the mammalian BAM system for both small payload flight experiments, and longer term space station studies.

Tissue culture conditions were developed for generating three dimensional mammalian muscle organs (organoids or bioartificial muscles, BAMs) from either primary murine skeletal myoblasts or a murine myoblast cell line C2C12 stably transduced with the gene for recombinant human growth hormone (rhGH). A simplified growth chamber was designed with the gross geometry of a skeletal muscle whereby the mononucleated muscle myoblasts could be cast in an extracellular matrix gel. Mechanical tension placed on the matrix-embedded cells during their fusion and differentiation oriented the newly formed myofibers longitudinally from end to end in the BAMs. An enriched media containing numerous growth factors was developed for the long-term maintenance (3 to 4 weeks) of these mammalian BAMs. Task 1 of the project was therefore accomplished during the first year. These BAMs have been utilized for cellular and molecular studies on tension/gravity regulation of muscle growth. Total cellular protein, myosin heavy chain content, and rhGH output are significantly reduced with tension release of the BAMs (Task 2). The thirty day survival of BAMs in modified bioreactor cartridges of the Shuttle Space Tissue Loss Module was accomplished during the Project's second year (Tasks 3 & 4) and will serve as a muscle wasting model for future small payload Shuttle flight experiments. Longer duration (60 - 90 day) studies with the BAMs will be possible in the Cell Culture Unit under development for the International Space Station. Implantation of rhGH-secreting BAMs into syngeneic animals has been recently found to be an

excellent long-term (3 months) cellular delivery platform for this anabolic growth factor (Task 5). Implantation of BAMs secreting 3 to 5 μg rhGH/day *in vitro* into hindlimb unloaded mice significantly attenuated plantaris and soleus muscle atrophy by 41% to 55%, respectively. In contrast, animals receiving daily injections (1mg/kg/day) did not show significant attenuation of muscle atrophy. These results support the idea that cell based delivery of rhGH is more effective in treating muscle wasting disorders than currently available injection techniques.

While the primary goal of this project is to understand and treat space travel-induced skeletal muscle atrophy, the results from these studies may have applications for several skeletal muscle wasting disorders on Earth. These include the severe muscle wasting observed in paralyzed patients and in the frail elderly, both of which partially respond to the increased tension associated with exercise and physical therapy. By better understanding the interactions of growth factors and mechanical tension, optimization of physical therapy could be optimized for increased patient mobility and independence. In addition, the potential exists for the use of the techniques developed as part of this project to tissue engineer human skeletal muscle BAMs containing foreign genes which code for a wide range of therapeutic bioactive molecules such as growth hormone, insulin, erythropoietin, tyrosine hydroxylase, and Factor IX. Implantation of these BAMs would be useful in the treatment of such Earth-based disorders as growth retardation, diabetes, renal failure, Parkinson's disease, and hemophilia, respectively. The feasibility and effectiveness of such tissue engineered muscle gene therapy techniques has been validated in animal models during the second and third years of the project.

FY97 Publications, Presentations, and Other Accomplishments:

Payumo, F.C., Kim H., Sherling, M., Smith L., Keeping, H.S., Valentini, R., Drozdoff V., and Vandenburg, H.H. "Expression of recombinant human bone morphogenetic protein-6 in C2C12 myoblasts." (Abstract) *Artif. Organs*, 21, 496 (1997).

Powell, C., Shansky, J., and Vandenburg, H.H. "Engineering of human bioartificial skeletal muscle for delivery of therapeutic molecules." (Abstract) *Artif. Organs*, 21, 496 (1997).

Shansky, J., Del Tatto, M., Chromiak, J., and Vandenburg, H. "A simplified method for the *in vitro* tissue engineering of skeletal muscle organoids." *In Vitro Cell. Dev. Biol.*, (In Press).

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Vandenburg, H.H., Chromiak, J.A., Shansky, J., and Del Tatto, M. "Effects of space travel on cell metabolism and protein turnover of skeletal muscle cells." (Abstract) *Am. Soc. Cell. Biol. Mtg. - Special Session*, H59 (1996).

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Effect of Gravity on Energy Expenditure by Rats

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Funding:

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Initial Funding Date: 1997	Expiration: 2000
Students Funded Under Research: 2	Post-Doctoral Associates: 0
FY 1997 Funding: \$0	

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Responsible NASA Center: Ames Research Center

Task Description:

The available data on the relationship between energy expenditure and gravity is conflicting, with some studies suggesting an increase and others a decrease. The objectives are to (i) test the hypothesis that energy expenditure of rats increases as the gravitational force increases; (ii) identify hormonal changes in urine which may be indicative of metabolic variations contributing to changes in energy expenditure due to alteration in gravity; and (iii) develop a methodology which will be capable of measuring the resting and activity energy expenditure rate and endocrine status of rats on the space station. These goals will be accomplished by using the double labeled water expenditure ($^2\text{H}_2\text{ }^{18}\text{O}$) method to measure energy expenditure rate and the excretion rates of hormones of rats over a 7-day period in altered gravity.

Four experiments are involved. Experiment 1: Development of the methodology for determining the BMR and energy costs of activity in rats at 1-G. With suitably timed urines, the energy expenditures and excretion of hormones between different pairs of points will be different reflecting differences in the proportion of time the rat is active and resting. The active:inactive time spent by the rats will be determined by video monitoring. The relevant equation will be solved for the energy costs of activity and rest. Experiment 2: Determination of how energy expenditure varies with G in the range 1- to 2-G. Five groups (16 rats per group) will be run. The groups are: (1) 1.5-G; (2) 2-G; (3) a Coriolis control on the 24-ft NASA-ARC centrifuge; (4) 1-G pair-fed control; and (5) 1 g normal control. Experiment 3: Investigation of the effect of angular momentum on energy expenditure. Five groups of rats will be run at 2-G, but the groups will differ in the distance the cages are placed from the fulcrum of the centrifuge. The groups are: (1) 3 ft; (2) 6 ft; (3) 9 ft and synchronous caged controls; (4) pair-fed; and (5) normal. Experiment 4: Role of glucocorticoids in metabolism during hypergravity. This will be assessed in adrenalectomized animals with replacement to basal corticosterone levels. The groups are sham operated and adrenalectomized in 1- and 2-G.

Funding for this task was not received until after April 1, 1997. A cooperative agreement has been initiated with UMDNJ. ACUC approval of a new protocol was obtained and studies as to the effects of 2-G hypergravity initiated. Two papers have been submitted to journals.

Metabolism is closely coupled with the work performed. Work is the distance a mass is moved. With increases in gravity the mass of an animal is increased. If activity (distance moved) is maintained, there should be a proportional increase in metabolism. This does not appear to be true suggesting changes in efficiency of movement and alterations in energy sources. The proposed work offers insights how these changes may occur, with possible improvements in performance of work at set energy levels.

FY97 Publications, Presentations, and Other Accomplishments:

Baer, L.A., Corbin, B.J., and Wade, C.E. "Alterations of body mass gain of neonates (P7 & P14) during centrifugation." *FASEB J.*, 11:A97 (1997).

Moran, M.M., Corbin, B.J., Grindeland, R.E., Roy, R.R., and Wade, C.E. "Circadian hemodynamic responses to acute hindlimb suspension." *FASEB J.*, 11:A97 (1997).

Ortiz, R.M., Wang, T.J., Corbin, B.J., and Wade, C.E. "Renal responses to centrifugation in rats." *FASEB J.*, 11:A86 (1997).

Wang, T.J., Ortiz, R.M., Corbin, B.J., and Wade, C.E. "Influence of altered mass loading on testosterone excretion and testicular mass." *FASEB J.*, 11:A30 (1997).

Permeability and Gene Expression in Brain Endothelial Cells Exposed to Shear Stress and Differential Pressure

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Funding:

UPN/Project Identification: 199-40-21-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 1

FY 1997 Funding: \$141,000

Responsible NASA Center: Johnson Space Center

Task Description:

One of the objectives of the Space Biology Program is to "determine the effects of the interaction of gravity and other environmental factors on biological systems." Translocation of fluid from the lower extremities to thoracic and cephalic regions upon exposure to microgravity is a well documented event. However, limited data are available concerning the influence of this headward fluid redistribution on the blood brain barrier. This cephalic fluid shift would be expected to induce mechanical stresses in the endothelial cells that make up the blood brain barrier. Mechanical stresses on a cell can be divided into two components: those that are tangential to the cell surface known as shear stress and those oriented perpendicular to the surface of the cell termed normal stress. We hypothesize that human brain-derived microvessel endothelial cells will respond to increasing mechanical stress by altering both hydraulic conductivity and the macromolecular permeability of a cell monolayer. In addition, we hypothesize that the effects of these forces are modulated by differential gene expression. The effect of both of these stress components will be studied independently and concurrently in an *in vitro* model using brain microvessel endothelial cells. Shear stress will be produced by flowing fluid across the surface of the cells, and normal stress will be induced by a hydrostatic pressure gradient across the cells. The effects of these mechanical stresses will be assessed by quantitative changes in hydraulic conductivity and macromolecular permeability. Techniques of differential and subtractive hybridization will then be used to isolate novel genes that are transcriptionally altered under the influence of these mechanical stresses. These studies will identify those genes that are responsive to shear and normal stresses in the blood brain barrier endothelial cells and provide insight into the molecular mechanisms that are associated with the fluid redistribution during the initial phases of microgravity and upon return to a normal gravitational environment.

The effect of shear stress on the levels of prostaglandin H synthase (PGHS) levels in primary human umbilical vein endothelial cells (HUVEC) were studied by exposing the cells to shear stresses of 4, 15, and 25 dyn/cm² for up to 24 hr using a parallel plate flow apparatus. Changes in PGHS1 and PGHS2 protein levels were quantitated by immunoblot analysis using two polyclonal antibodies specific for PGHS1 and PGHS2. Changes in the amount of PGHS1 and PGHS2 in the cells occur within 10 min of when shear stress is first applied to them. Prostaglandin H synthase 1 protein levels in cells subjected to shears stresses of 4, 15, and 25 dyn/cm²

increase 4.3, 1.91, and 2.18 fold over protein levels in stationary control cells. In contrast, after 10 minutes of shear stress, the amount of PGHS2 in the cells increase only slightly at 4 dyn/cm², (1.29 fold), decrease at 15 dyn/cm² (0.86 fold) and remained constant at 25 dyn/cm². After 24 hr of shear stress dramatic increases in PGHS1 levels take place for shear stresses of 15 dyn/cm² (18 fold) and 25 dyne/cm², (11 fold). Whereas, at 24 hr PGHS2 protein levels remain unchanged. At 4 dyn/cm² there is a 2.6 fold increase in PGHS1. Indicating that there maybe a sudden increase in PGHS1 levels at 4 dyn/cm² when HUVECs are first subjected to the pressure followed by a decrease as the cells continue to be cultured in the presence of the shear stress. At 4 dyn/cm², changes in PGHS2 protein levels are only slightly altered increasing 1.29-fold after 24 hr.

These data are quite interesting since of the two genes, PGHS2 has been considered to be a regulated gene and PGHS1 has been thought to be a constitutively expressed gene. The PGHS1 promoter is similar to that of other constitutively expressed genes in that it lacks a TATA box and has multiple transcription initiation sites. However, there is a putative shear stress regulatory element in the PGHS1 promoter. Preliminary results using quantitative RT-PCR indicate that PGHS1 transcript levels increase approximately 4 fold after 24 hr of shear stress at 15 dyn/cm². In comparison, the PGHS2 gene's promoter does not contain a putative shear stress regulatory elements and there is only a 1.5 to 2 fold increase in mRNA after 24 hr of shear stress at 4, 15, and 25 dyn/cm². The 4 fold increase in PGHS1 mRNA levels at 4 dyn/cm² after only 10 minutes of shear stress and the large increase in protein level at 24 hr compared to the change in the gene's transcript levels suggest that shear stress may have an effect not only on the amount of PGHS1 mRNA present in the cell but also on the rate at which it is translated into protein. These data are unique in that it is the first demonstration that a gene may be regulated by shear stress at the translational level.

Our studies will increase our understanding of cephalid fluid redistribution relevant to entry into and recovery from the microgravity environment. The ground-based benefit of these studies will enhance our understanding of the blood-brain barrier (BBB) in hypertension and cerebral trauma. Our studies focus on the physical forces at a cellular and molecular level. Using an *in vitro* model system of the BBB offers advantages of decreased complexity and a more experimentally accessible environment and enables the study of shear and hydrostatic pressure effects, independently and together, at the cellular/molecular level. With this level of understanding, the role of shear stress and hydrostatic pressure mechanisms in fluid redistribution will be clarified. Although the effect of shear stress has been studied with some vascular endothelial cells, this study is the first to examine the effects of shear stress on the specialized endothelial cells that make up the BBB. Brain microvessel endothelial cells differ biochemically from those in other vascular endothelia. Although similarities exist, brain microvessel endothelial cells may be modulated differentially by shear and pressure forces as compared to other endothelial cells. In addition, the effects of hydrostatic pressure on endothelial cells, blood brain barrier-type or other cells, have not been examined in detail. Therefore, the studies described offer unique opportunities to examine the effects of these physiologic forces on gene regulation in the specialized endothelial cells of the BBB. This knowledge about the BBB may be useful in developing treatments for cerebral trauma/edemas as well as for space motion sickness, or understanding the effects of hypertension. Therapeutic approaches may be developed based on a better understanding of the permeability properties of the BBB. Alternative physical or pharmacologic methods may be indicated as a result of this research.

Effects of Silver and Other Metals on the Cytoskeleton

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Funding:

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Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 2

Post-Doctoral Associates: 1

FY 1997 Funding: \$ 132,820

Task Description:

Directly or indirectly, trace concentrations of silver ion (Ag^+) stabilize microtubules, as does taxol, an effect with major consequences for cellular shape changes and development. Polymerization of microtubules is gravity sensitive, so trace amounts of Ag^+ may alter cellular ability to respond to gravity. If Ag^+ electrolysis is used to purify water on NASA space vehicles, plants and animals/astronauts will be exposed continuously to Ag^+ , a regimen with unknown cellular and developmental consequences. Fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta*, are the cells in which the effects of Ag^+ on microtubules were discovered. They distribute visible cytoplasmic contents according to gravity and contain cytoplasmic morphogenetic determinants for heart development. The objectives are to determine if the effects of Ag^+ , Au^{3+} (of biosensor relevance), or Gd^{3+} (inhibitor of some stretch activated ion channels) on the cytoskeleton (in the presence and absence of mechanical loading) will affect cellular responses to gravity.

To begin to extend the observations from *Ilyanassa* cells to examine the effects of Ag^+ on a variety of microtubule-dependent phenomena in vertebrate cells, two additional types of studies were initiated during the past year. First, the effect of Ag^+ on the normal increase in cell numbers in human cells *in vitro* was studied. Second, the effect of Ag^+ on the outgrowth of neurites and glial cells from dorsal root sensory ganglia of chicken and quail embryos was studied.

We have continued to study the effects of Ag^+ on the normal cellular shape change displayed by the fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta*. We have demonstrated previously that colchicine and nocodazole cause disappearance of microtubules. We have also demonstrated previously that exposure of fertilized eggs to Ag^+ causes each cell to form a very long, constricted neck of cytoplasm ("Ag-neck") which contains a very large (abnormally large) number of microtubules; this effect is very reminiscent of the effects of treating these cells with microtubule-stabilizing agents (e.g., taxol or hexylene glycol). This correlation led to the hypothesis that treatment of the cells with Ag^+ caused the formation of the dramatic cell shape (Ag-necks) by stabilization of microtubules. During the past year, we have therefore tested this hypothesis by determining whether Ag^+ could still cause Ag-necks to form in the absence of microtubules. We therefore treated fertilized eggs with nocodazole to induce microtubule depolymerization and inhibit formation of the normally very tight (but short) cytoplasmic neck. We then observed such cells in the presence and absence of Ag^+ . Results indicate

that even in the presence of nocodazole (absence of microtubules), Ag^+ can cause an enhanced constriction and elongation of the cytoplasmic neck. These results suggest that Ag^+ can cause an enhanced constriction by activating the activity of elements such as microfilaments and that its enhancement of microtubule numbers may be a secondary effect. Thus, we have prepared peptide antibodies to *Ilyanassa* myosin to use for immunolocalization of that protein during cellular shape changes in control and Ag^+ -treated eggs.

We have also studied the effects of a variety of concentrations of Ag^+ on the normal increase in cell numbers that occurs upon inoculation of cells *in vitro*. We found that continuous exposure of human cell lines to Ag^+ in the micromolar range suppresses cell proliferation.

We have also found that continuous exposure of chick or quail dorsal root sensory ganglia *in vitro* to Ag^+ in the micromolar range suppresses the outgrowth of both neuronal growth cones and of their associated glial cells (fibroblastic morphology).

Silver metal (Ag^0) and ions are being used on Earth for many applications and there is a pervasive opinion that, although Ag^+ is toxic for microorganisms, it is harmless to humans and other eukaryotic organisms. Silver-purified water is increasingly available for drinking, bathing, and swimming pools. In addition, many health-food stores in the U.S. are selling increasing varieties of "colloidal silver" as a health food supplement to "destroy all the pathogenic microorganisms or infections in your body" and to "gradually build your immune system." However, physicians have warned recently of the long-term danger of consuming Ag-containing solutions for long periods of time can cause a generalized deposition of Ag^0 in the skin and mucous membranes which remains permanently as grey depositions (Ag^0 metal), a condition known as argyria. Moreover, many organ systems and specific enzymes are inhibited by Ag^+ at concentrations equivalent to those being used in the applications described above. Our research represents a focused attempt to understand the molecular mechanism(s) involved in the toxic effects of Ag^+ on animal cells.

FY97 Publications, Presentations, and Other Accomplishments:

Conrad, A.H., Koo, S.J., Hebert, G.L., and Conrad, G.W. "Expression and localization of myosin in fertilized eggs and adult tissues of *Ilyanassa obsoleta*." Bull. Mt. Desert Isl. Biol. Lab., 36:11-13 (1997).

HOX Genes and Pattern Formation in Mouse Limb Buds

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Funding:

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Students Funded Under Research: 0	Post-Doctoral Associates: 0
FY 1997 Funding: \$0	

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The embryonic limb is an excellent model for morphogenesis, but early development of the limb has not been studied in space. Our centrifuge studies of *in utero* and *in vitro* limb development found alterations in pattern in centrifuged limbs, indicating that certain homeobox genes might be affected (e.g., MSX 1 and MSX 2, involved in patterning along the proximo distal axis of the limb, and the multigene cluster HOX D, involved in anterior-posterior patterning). In this study, we propose to examine the effect of excess gravity exposure on the expression of homeobox genes in 11 d embryonic mouse limb buds developing in culture, and to relate changes in gene expression to changes in formation of precartilaginous blastemata and cartilage anlage. We hypothesize that the major effect of excess gravity exposure on the expression pattern of HOX genes is temporal, delaying the sequence of events, resulting in asynchronies in tissue interaction and induction which result in altered pattern formation. Since expression of HOX genes affects processes important in limb development, such as cell-cell interactions (adhesiveness) and formation of gap junctions, a portion of our project will examine those parameters. We will also study selected extracellular matrix molecules (collagens Type I and II, and fibronectin) that are critical in the developmental process, and whose expression is strictly controlled in time and space. This study is the first to address limb development, HOX gene expression, and patterning in altered gravitational conditions.

Because expression of HOX genes occurs very early in limb development, in the main occurring prior to the time that limbs can be cultured successfully, a number of experiments to date have been concerned with developing techniques to allow very young (< 11 d.) limb buds to be cultured. To culture 10 and 11 d. limbs, portions of the flank were included, since outgrowth of the limb is due to cells coming from the flank. Staining with methylene blue to allow identification of developed limb elements found that development was good only in the proximal regions of the limb, and that development of the wrist/ankle and hand/foot in these studies was never very good. A significant amount of cartilage did form in the distal region, but pattern could not be discerned due to lack of outgrowth of the epidermis. Since the AER was present at excision, the lack of

outgrowth is not due to absence of the AER, although some significant interaction of the AER with the underlying mesenchyme could be affected by excision and culturing of the limb.

We also assessed the published methods for storing collected limb buds in the cold for up to a week prior to culture, but were not very successful in our hands. Limb buds stored in the cold in serumless medium for 2, 4, 5, or 7 days exhibited some growth and differentiation, but never reached the levels of controls. The inability to grow 10 and 11 d limb buds will not allow us to use some of the probes we originally planned on using, i.e., HOXD. But MSX1 probes have been obtained, and preliminary *in situ* carried out.

The early stages of limb organogenesis are sensitive to interruption by teratogenic agents, or certain mutations, and interference with these events has extreme consequences on further development. Well-known examples of teratogenic effects include those of thalidomide, and retinoic acid; mutations include various dyschondroplasias ranging from short limbs to lack of limbs through lethal skeletal defects. Thus, the question of limb malformation is a significant health problem on Earth.

HOX genes have been found to direct the early stages of limb development. The role of HOX genes in limb development is being defined in many labs using techniques such as grafting, mutations, exposure to teratogens (retinoic acid), and viral over-expression. Our lab uses gravitational changes as another tool to examine this role. If gravitational changes alter the early stages of limb development (i.e., when the pattern is being laid down), more serious developmental changes upon exposure to altered gravity can be expected than if the effect is primarily on growth and maturation of the formed elements. Previous studies in this lab have shown that limb pattern is indeed affected by exposure to excess gravity, and the current study is expected to show that this effect is due to an effect of gravitational changes on the expression of HOX genes.

FY97 Publications, Presentations, and Other Accomplishments:

Duke, J. and Montufar-Solis, D. "Tissue engineering in space: Producing cartilage for repair of bones on Earth." Abstracts - Fifteenth Annual Houston Conference on Biomedical Engineering Research. Houston, TX (February 14, 1997).

Duke, J., Arizpe, J., Montufar-Solis, D., D'Souza, R., and Mackenzie, I. "Cartilage differentiation and endochondral ossification in implanted embryonic limb buds." Presented at the Bones and Teeth Gordon Conference, Meridan, NH (July 6 - 11, 1997).

Duke, P.J. "New views, new vistas in the Life Sciences: ISS and beyond." (Invited paper for a Plenary Lecture). Presented at the In Space '96 Conference, Japan Space Utilization Promotion Center, Tsuba, Japan (November 11 - 12, 1996).

Duke, P.J. and Montufar-Solis, D. "The chondrocyte's response to load." Presented at the International symposium for frontiers of biological science in space. Tokyo, Japan (February 8, 1997).

Duke, P.J. and Montufar-Solis, D. "The differentiation of chondrocytes in altered gravity." Presented at the In Space '96 Conference, Japan Space Utilization Promotion Center, Tokyo, Japan (November 11 - 12, 1996).

Duke, P.J., Montufar-solis, D., Grant, R., and Martin, G. "Use of a NASA bioreactor in engineering tissue for bone repair, a preliminary report." Presented at the Eighth Annual University of Texas Dental Branch Research Day, Houston, TX (April 25, 1997).

Duke, P.J., Montufar-Solis, D., Williams, N., and Kriswell, D. "Cephalometric changes in tail-suspended mice and the effect of a skeletal muscle transgene." Presented at the American Society for Gravitational and Space Biology annual meeting, Charlotte, NC. (Abstract) ASGSB Bull. 10, 58 (October 25, 1996).

Gravity and the Regulation of a Central Growth Factor Pathway

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Funding:

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Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$ 131,640

Task Description:

This proposal is based on preliminary studies with both hypergravity and microgravity which indicate that the production of a physiologically important growth factor, epidermal growth factor (EGF), is a gravity sensitive system. Using the submandibular salivary gland (SSG) as indicator tissue for EGF production, we have observed a statistically significant reduction in intracellular EGF levels in mice exposed to excess gravity and an increase in intracellular EGF levels in space flight animals (rats) suggestive of secretory impairment (STS-54 mission). It remains to be determined whether the observed effects are at the level of EGF synthesis (transcription/translation), or storage or secretion (e.g., lack of storage due to increased secretion or protein degradation). Important to both NASA's Space Biology and Space Physiology programs are several well-documented *in vivo* EGF functions including a role in wound healing, maintenance and cytoprotection of the gastrointestinal tract, reproduction, bone metabolism, calcium homeostasis, and modulation of fluid balance and nervous system function. Because of the relevance of EGF actions to human physiology, the aims of the proposed studies are: (1) to systematically document our preliminary observations with a variety of EGF-producing organs and to determine whether gravitational effects on EGF are reversible; (2) to assess the mechanisms(s) whereby altered gravity affects EGF production *in vivo*; and (3) to determine whether simulated gravity or excess gravity alters EGF production via direct effects on the cells. It is hoped that the proposed ground-based studies will serve as a prelude to flight experiments with both cells in culture and animals. To our knowledge, this is the first study to address the hypothesis that gravitational fluxes affect a central growth factor biosynthetic pathway (either by direct effects on cells or indirectly via modulatory molecules) whose deregulation could lead to abnormalities in a wide variety of physiologically important processes. Given that therapeutic approaches with EGF have proven to be possible, knowledge derived from the proposed studies may help develop approaches to reduce health risks associated with long-term space missions.

This project was funded at the end of fiscal year 1997, thus only a limited number of experiments were initiated during this period. The goal of these experiments was to establish whether changes in gravity directly affect cellular production or secretion of EGF. Primary cultures from an organ that produces vast amounts of EGF (salivary epithelium) were successfully established and expanded using a NASA-designed rotating wall vessel bioactor. Dissociated cells were seeded either on collagen-coated Cytodex-3 microcarrier beads or within collagen gels. Large 3-dimensional structures (3-4 mm in diameter) were generated under both seeding

conditions after 2 weeks of culture. However, the growth characteristics of these cultures were different. Histological examination demonstrated that cells growing on Cytodex beads attached well to the collagen layer and grew as a multilayer solid mass with cells closely apposed to one another. Scanning electron microscopy demonstrated that cells in these multilayer structures possessed numerous microvilli. Within one week of culture, cells seeded within collagen gels generated instead extensive ductal-like extensions throughout the matrix. Formation of multilayers was rarely seen in these cultures, although lumens were occasionally visible. Control collagen gel cultures in 60 mm dishes were contrasted to bioreactor cultures within collagen gels. The simulated microgravity conditions of the bioreactor enhanced generation of ductal-like extensions both regarding time of appearance and quantity. Levels of EGF in the bioreactor and control 1-G cultures are presently being examined.

The significance of the proposed studies encompasses both basic and clinical issues. EGF is a crucial regulator of diverse biological processes; alterations associated with its production or secretion conceivably can provide an underlying mechanism for a number of space flight-induced abnormal responses. Our preliminary studies have indicated that EGF production, storage, or secretion *in vivo* is affected by both excess gravity and space flight. However, we do not know whether gravitational fluxes directly affect cellular production of EGF or indirectly achieve this effect via systemic forces such as alterations in hormone levels. The proposed studies are aimed at clarifying these issues. Documented *in vivo* functions of EGF include a role in maintaining gastric and oral mucosal integrity and cytoprotection, stimulating epidermal wound healing, renal tissue repair, bone resorption, modulating fluid balance, and nervous system and reproductive functions (e.g., oocyte maturation, lactation, and spermatogenesis). Thus, deregulation of this biosynthetic pathway can bring about alterations in various physiological responses. Since therapeutic approaches with EGF are being explored and are proving to be possible, knowledge derived from the proposed studies could be used to implement protocols to minimize potential health risks associated with long-term human space missions.

*Lineage Analysis of Axis Formation Under Novel Gravity***Principal Investigator:**

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Funding:

UPN/Project Identification: 199-40-27-22

Solicitation: 93-OLMSA-07

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$147,356

Task Description:

Recent intriguing work by Cooke (1986) and Neff et al. (1993) suggests that there are subtle developmental changes in the *Xenopus laevis* embryos subjected to novel gravitational fields. These changes include the position of the third cleavage plane, the dorsal lip of the blastopore, and also the size of the head and eyes. However, compensation occurred later in development, so that by the tadpole stages there is no apparent difference between experimental and control embryos. How these early morphological changes are corrected is not clear. Through this project, we plan to determine whether the distribution of cytoplasmic morphogenetic determinants, and thus the developmental fate of blastomeres, is altered by novel gravitational fields by either tilting them or rotating them in a horizontal clinostat. We then plan to compare the control and experimental embryos with respect to blastomere fate (by lineage tracing with fluorescent dextrans), blastomere commitment and autonomous differentiation potential (by transplantation and culture), and distribution of cytoplasmic morphogens (by *in situ* hybridization). These three approaches, when applied in tandem, will provide a definitive test of the hypothesis that the distribution of cytoplasmic morphogenetic determinants and thus the developmental fate of blastomeres can be altered by novel gravitational fields.

In the fiscal year of 1997, we completed the Experiment II and started the Experiment III as proposed in the grant proposal. In addition, we performed some pioneer experiments related to the current project but not originally proposed.

1) Experiment II was designed to investigate the change in the autonomous differentiation capabilities of cultured blastomeres and the change in the ability of blastomeres to signal and respond during early inductive events. Normally, at the 16-cell stage, only dorsal blastomeres are able to elongate and express dorsal differentiation in explant culture. However, in 90° rotated embryos, the dorsal animal blastomere lost the ability to elongate or to express dorsal markers, while the ventral vegetal blastomere assumed these abilities. More importantly, the vegetal blastomeres in 90° rotated embryos and the dorsal blastomeres in clinostat-treated embryos increased their ability to elongate and express dorsal markers. It suggests that 90° egg rotation or microgravity may reorganize the cytoplasmic components, bringing localized dorsal determinants together, and thus facilitating the expression of dorsal fate in the blastomere. To study the possible molecules brought into the blastomeres to enhance their dorsal fate expression was proposed in the grant competitive renewal.

2) In Experiment II, we also used cell transplantation to examine the effect of gravity on the inducing and responding abilities of blastomeres. As expected, these two cell properties are changed in 90° rotated embryos. That is, the dorsal-inducing ability of normal dorsal blastomeres is adopted by ventral vegetal blastomeres in 90° rotated embryos. On the other hand, the vegetal blastomeres changed their ability to produce retina in rotated embryos. These results have been reported at the Developmental Biology annual meeting and a complete manuscript has been written.

3) We have started Experiment III. It includes the *in situ* hybridization studies on the distribution of putative dorsal determinants, such as Vg1 mRNA, in normal and gravity-treated embryos. Thus far, the study on Vg1 mRNA redistribution in normal, 90° rotated and microgravity-treated embryos has been conducted. The results demonstrate that Vg1 mRNA partially shift position under the effect of gravity. Next, we will study the redistribution of Vg1 mRNA in the embryos subjected to hypergravity and to relate the redistribution of Vg1 mRNA and the cell fate changes of the blastomeres we observed earlier.

4) Recent studies suggested the importance of interaction between dorsal determinants on the dorsal differentiation. The idea of determinants interaction may explain why the dorsal differentiation of blastomeres is enhanced in the 90° rotated embryos we observed. Therefore, we centrifuged normal and UV-treated embryos to test the possibility of dorsal determinant interaction in dorsal differentiation. Some interesting results have been obtained and analysis is under way.

This project will investigate the early changes in development caused by gravitational alterations at the cellular and molecular levels. It will define time points of exposure from which embryos can recover and lead to studies of time points after which embryos cannot regulate. Defining this critical developmental window will contribute to NASA's research goals by providing basic information of importance for attempts to raise animals in space.

FY97 Publications, Presentations, and Other Accomplishments:

Huang, S. and Wang, H.Z.P. "Cell fate change in axis tilted xenopus embryos is the result of the cytoplasmic reorganization under the effect of gravity." ASGSB Bull., 36 (1996).

Huang, S., Johnson, K.E., and Wang, H.Z.P. "Blastomeres show differential fate changes in 8-cell *Xenopus laevis* embryos that are rotated 90° before first cleavage." Dev. Growth & Differ., (In Press).

Huang, S., Wang, H.Z.P., Johnson, K.E., and Wei, W.L. "Cell properties in early *Xenopus* embryo are altered by novel gravitational conditions." Develop. Biol., (suppl.) 186:A170 (1997).

Vestibular Ontogeny, Adaptation and the Effects of Gravitational Loading in the Rat

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Funding:

UPN/Project Identification: 199-40-27-05

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 2000

Students Funded Under Research: 4

Post-Doctoral Associates: 0

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The purpose of the proposed research is to test the following hypotheses in the rat (*Rattus norvegicus*): 1) gravitational loading can significantly alter the course of vestibular functional development; and 2) vestibular functional adaptation occurs in the periphery in response to chronic gravitational loading or unloading and such adaptive plasticity can be characterized using vestibular responses to pulsed linear acceleration. To test these hypotheses, vestibular evoked potentials will be recorded using linear jerk stimuli in rats raised at 1G and chronic 2-G centrifugation. Vestibular response thresholds, latencies, and amplitudes will be characterized during normal ontogeny and these normal patterns will be compared to those observed in rats raised under the influence of 2-G centrifugation.

The proposed studies will fulfill three specific objectives: 1) the ontogeny of vestibular responses to pulsed linear acceleration will be characterized in the rat for the first time; 2) the developmental patterns of normal and centrifuged animals will be quantitatively compared and evaluated for evidence of differences; and 3) adaptive changes in vestibular function with increasing or decreasing acceleration steps will be evaluated in neonate and young adult rats. The knowledge gained from this investigation will lead to a better understanding of the role of gravity in peripheral vestibular function and development. This goal is consistent with the research emphases of the Developmental Biology component of the Space Biology program. Furthermore, the results will improve our understanding about how the inner ear develops normally and what factors can modify development to produce abnormal growth and function in animals, including humans. Finally, the results will complement future space-flight experiments proposed to evaluate the effects of microgravity on mammalian vestibular development.

The project was initiated June 15, 1997. The nature of the VsEP in rats is under study. Compound action potentials of the vestibular evoked potentials (VsEP) in turn are being used to characterize normal development in rats raised at 1-G. Initial findings indicate that the adequate stimulus in the rat is kinematic jerk. This is consistent with previous findings in the bird.

The results of work completed to date suggest that the gravity receptors of developing birds and mammals are dynamic in that they exhibit an increase in sensitivity during maturation. There may be natural environmental factors that can alter these maturational profiles. One such factor could be gravity itself since it is a natural stimulus during ontogeny. Does normal vestibular development require Earth's 1-G environment? Gravity is markedly decreased during space flight and the vestibular system of developing embryos subjected to the microgravity environment might develop abnormally (Jones 1992; Jones et al., 1991, 1993; Fermin et al., 1996). Gravitational field strength can also be increased using a centrifuge. Although these issues have been studied recently in the bird, little is known regarding the possible role of environmental factors in mammalian peripheral vestibular receptors. The current research will use VsEPs to evaluate development in mammals (rats). Our studies of the nature of mammalian VsEP suggest that, like the bird, VsEPs in the rat reflect the activation of a subset of gravity receptor neurons, in particular those signaling linear jerk. This knowledge further improves our understanding of vestibular responses and our ability to characterize the developing vestibular system. It is important that we clearly define the nature of the VsEP functional test and establish that it is, in fact, a vestibular test for all ages studied. This has been accomplished in the bird, but remains a critical focus in the current studies in the rat. These represent significant steps toward our goal of evaluating the role of gravity in the ontogeny of gravity receptors. Moreover, these studies provide insights that may lead to the successful application of the new vestibular test in the diagnosis of the dizzy human patient.

FY97 Publications, Presentations, and Other Accomplishments:

Bothwell, M. and Jones, T.A. "Mammalian vestibular evoked potentials: The influence of peak acceleration amplitude under iso-jerk conditions." Am. Acad. Otolaryngol-Head & Neck Surgery, Presentation and Abstract, Annual meeting, San Francisco, CA (1997).

Ultrastructural, Neurochemical and Developmental Responses to Hypergravity

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-40-17-13

Solicitation: 96-OLMSA-01

Initial Funding Date: 1997

Expiration: 1999

Students Funded Under Research: 2

Post-Doctoral Associates: 0

FY 1997 Funding: \$78,875

Task Description:

The broad long-term objective of research in my laboratory is to understand sensory processing in the vestibular system. Specific aims of the proposed work are: 1) to provide a detailed ultrastructural analysis of regional variations in cellular and synaptic architecture in the utricular macula of the developing and adult mammal, and 2) to study the influence of hypergravity conditions on cellular and synaptic activity in the hair cell and upon the nitric oxide synthase efferent innervation of the otolithic maculae. In previous work, we have described regional variations in synaptic innervation of the crista. Similar studies are planned for the otolithic endorgans in the developing and adult mammal. These studies will provide baseline data for future flight studies. In addition to this, we are examining the effect of hypergravity conditions upon certain features of synaptic innervation. Some of the cellular and synaptic features that vary regionally are likely to be a physiological response to stimulation.

We have also recently described the presence of a new transmitter in the vestibular efferent system, nitric oxide. There is evidence that it has a specific effect upon type I hair cells. We will be investigating the hypothesis that it can respond rapidly and upregulate in response to changes in linear acceleration or hypergravity.

What has been accomplished thus far?

In the five months since this grant has been active, we have made progress toward our first goal, a detailed description of the normal synaptic innervation of the utricular macula in the developing and adult animal. We chose to examine the developmental stages of PD0 (day of birth), PD4, PD7, PD10, and PD28, to correlate these data with our studies on the transition of hair cells from supporting cells, and subsequent type I and type II hair cell development (Lysakowski, Rüschi, and Eatock, *Soc. Neurosci. Abst.*, 22:1064, 1996).

Multiple samples were taken from each of the 5 postnatal stages. Dissector counts of synaptic ribbons were made as described previously in a study of the chinchilla crista (Lysakowski and Goldberg, *J. Comp. Neurol.*, 389: 413-443, 1997). These counts revealed that synaptic innervation in both type I and type II hair cells proceeds at an orderly rate.

Striolar hair cells contain more ribbons per hair cell than extrastricular hair cells at all stages, which parallels the gradually increasing production of mature hair cells in the striola compared to the extrastricular (Lysakowski et al., op. cit., 1996). Furthermore, the average number of ribbons per hair cell increases with each postnatal day,

from 5.7 (at PD4) to 6.3 (at PD7) to 9.1 (at PD10) to 25 (at PD28). Immature hair cells had fewer synaptic ribbons than mature hair cells at the same developmental stage. There was some tendency for type II hair cells to have more synaptic ribbons than type I hair cells, but this tendency disappeared as the animal approached maturity.

What questions have been answered?

One outstanding question has been answered in this short period. A previous study of synaptic innervation in cats showed that the numbers of synaptic ribbons in type I hair cells decreased 93% from birth to adulthood (Favre and Sans, *J. Neurocytol.*, 8:765-775, 1979). The results of this study have been very puzzling, since our studies in several species of adult crista (Lysakowski, *NYAS* 781:164-182, 1996) show that type I and type II hair cells in many vertebrate species contain similar numbers of synaptic ribbons. The results of Favre and Sans would seem to imply that newborn cats contained at least 10 times the numbers of synapses than those of adult cats. Our present developmental results are at variance with the results of their study. Comparing our results to the previous study by Favre and Sans, the differences may perhaps be explained either by an unusual species difference, or by our use of serial sections, or by our use of the disector method compared to their use of a ratio method of counting.

What new questions have arisen?

Clusters of synapses are common in hair cells of both types in the newborn mouse. "Hollow" synapses are also common. It remains to be determined whether these manifestations are merely immature forms of synaptic ribbons. Are they restricted to the striolar or central zone, a specialized portion of the sensory epithelium, as they are in the adult? Or do they represent an immature form that is widely distributed early on, and then restricted to the striola in the adult? In other words, does the striola exhibit more synaptic plasticity in the adult than the extrastriolar zone, and what are the implications of this for normal functioning of the sensory epithelium?

How does this fiscal year's progress affect future work on this task?

We will continue collecting data on the developmental portion of this task and will shortly resume the hypergravity experiments. Another aspect of our first goal, to determine the deleterious effects of hypergravity upon cytoskeletal elements in hair cells, now appears to be an effect of aging, since it was not replicated in our younger animals. We are, therefore, planning to use more aged animals to determine whether one particular subclass of afferents, such as striolar dimorphic units (the high-gain irregular afferents), are more affected by hypergravity than are their calyx unit counterparts (the low-gain irregular afferents) in the aged mammal.

This basic science research should yield a new understanding of two basic biological processes, namely the normal development, and the plasticity of the mammalian inner ear. Such information is important as man begins to inhabit space for longer durations. Ultimately, as man and animals begin to live and reproduce in space, it becomes important to know what effect microgravity will have upon the development of the inner ear in space. Without baseline information about what the normal developmental sequence of synaptic innervation is in the vestibular periphery, how can we hope to know how it is perturbed in space?

FY97 Publications, Presentations, and Other Accomplishments:

Lysakowski, A. and Ahmed, P. "Early postnatal development of synaptic innervation in the mouse utricular macula." (Abstract) ARO Midwinter Mtg. Absts. (In Press).

Lysakowski, A. and Goldberg, J.M. "A regional ultrastructural analysis of the cellular and synaptic architecture in the chinchilla crista ampullaris." *J. Comp. Neurol.*, 389, 413-443 (1997).

Altered Gravity and Early Heart Development in Culture

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Funding:

UPN/Project Identification: 199-40-27-24

Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 0

FY 1997 Funding: \$76,523

Task Description:

The effect of altered gravitational forces on the early development of the heart in the chick embryo will be examined using pre-cardiac explant tissues incubated in culture. The extracellular matrix glycoprotein fibronectin is known to be important in the heart development process. The goal of the proposed work is to obtain a new understanding of the effect of altered gravity on the production and deployment of fibronectin during the early development of the heart, and to determine whether or not these alterations lead to impaired cardiac myogenesis or morphogenesis. Previous experiments have shown that the microgravity of space flight delays the accumulation of contractile myofibrils in these tissues during an early sensitive period, and preliminary data suggest that coincident with this fibronectin may be greatly restricted.

Immunostaining, immunoassay, and electron and light microscopy will be used to determine, for precardiac explants developing under conditions of microgravity, unit gravity, and hypergravity, the following specific objectives: a) the overall tissue morphology, the immunolocalization of fibronectin, b) the measured accumulation and production of fibronectin, c) the ultrastructural characteristics, and d) the ability to develop spontaneous contractions. The proposed work aims to elucidate the mechanism by which gravity affects cells during their development in an experimentally accessible, well-characterized system, and it is relevant to NASA's mission in understanding the effects of gravity on animal development processes.

Precardiac tissue explants have been dissected out of chick embryos in pairs and cultured in a high aspect ratio bioreactor vessel (HARV) to simulate microgravity, or as controls at unit gravity. Explants in the HARV were rotated at 6 rpm to achieve continuous freefall. After 18 hours of culture at 38 degrees C, the explants were observed for tissue morphology and the development of contractions. They were then fixed and processed for immunolocalization of fibronectin using a monoclonal antibody. Sections of the explants were photographed, captured digitally, and analyzed with NIH Image, a public domain software program. Color thresholding and measurement of fibronectin staining parameters were carried out on a Power Macintosh 8100/80 computer. Exposure to microgravity did not alter the morphology or size of the explants. Three experiment groups with the HARV operating normally showed a significant difference in the number of explants that could carry out spontaneous contractions. A summary of all HARV experiments is given in Table 1.

Table 1. Comparison of development of spontaneous contractions in control and HARV-rotated chick embryo precardiac explants cultured in different circumstances.

<u>Category</u>	<u>Number of Control Explants and Percent Contracting</u>	<u>Number of HARV-Rotated Explants and Percent Contracting</u>
Kansas experiment, no stopping, agarose support	35/37 (95%)	1/37 (3%)*
HARV stopping, no agarose	65/89 (73%)	42/68 (62%)
HARV not stopping, noisy, no agarose	18/26 (69%)	15/22 (68%)
HARV, quieter, agarose support	38/45 (84%)	22/35 (63%)*
HARV, quieter, no agarose support	39/44 (89%)	24/40 (60%)*

*Significantly different from controls, $P < 0.005$

Immunostaining analysis showed fibronectin present in the basement membrane and mesenchymal regions of the control explants, but an apparent reduction in the amount of staining in the HARV-rotated, particularly in the basement membrane. Image analysis measurements showed no difference in the color thresholded total area of fibronectin staining calculated as a percent of section area ($P = 0.204$). However the length of fibronectin-immunostained basement membrane in the sections was significantly less in HARV-rotated explants ($P = 0.021$). The areas of HARV-rotated explants did not differ from controls.

The results indicate that altered gravity in the HARV had no effect on the shape and size of the explants, but did reduce the number of explants that developed contractions. Correlated with this is a reduction of linearly-arrayed fibronectin in basement membranes, suggesting some degree of failure of establishment of cell polarity.

Progress on immunoassays of the amount of fibronectin present in tissues, and on electron microscopic analysis is continuing.

Hypergravity experiments were conducted in July and August, 1997, at NASA-Ames Research Center. About 150 explants were dissected out and cultured in 96-well microtiter trays at G-forces of 2.0, 1.4, and 1.0 (controls), using the short-arm centrifuge. In addition, experiments have been carried using agarose as a support for the explants as well as without. The results for development of spontaneous contractions reveal a remarkable, almost absolute sensitivity to hypergravity, which was shown to virtually abolish development. Because of this, an extra control experiment was carried out during which the explants were centrifuged at the lowest possible speed, 40 rpm, which generated a calculated G-force of 1.025. At this G-force, development of spontaneous contractions was restored; however, it is not clear whether there was complete restoration, suggesting the possibility that vibrations may play a role. The results of the centrifuge experiments are displayed in Table 2.

Table 2. Precardiac explant culture in hypergravity, Short-arm Centrifuge, NASA-Ames Research Center, Moffett Field, CA.

<u>Gravity (G-force) /Substratum Support</u>	<u>Number of Explants Cultured</u>	<u>Number of Explants Contracting after 18 Hours of Culture</u>	<u>Percent</u>
1.0 / plastic surface	21	11	52%
1.0 / agarose support	49	41	84%
1.4 / agarose support	39	0	0
2.0 / plastic surface	22	0	0
2.0 / agarose support	8	0	0
1.025 / agarose support (vibration control)	9	5	56

Samples of hypergravity experimental and control explants have been fixed, processed, sectioned, and immunostained. However image capture and analysis of fibronectin staining is still underway. Samples of explants and medium from these experiments were also frozen and shipped back to UNI (Iowa) for immunoassay.

Still other samples were fixed and shipped to KSU (Kansas) for electron microscopy and assessment of myofibrillogenesis. These tasks are still in progress.

It is interesting that in both microgravity and hypergravity experiments we have seen some evidence for the possible effects of vibrations acting in the heart development process. Because vibrations might be perceived by cells through the same mechano-receptive apparatus as altered G-forces, perhaps this is not surprising. But it is particularly interesting that in the case of the HARV bioreactor, extra noise from the instrument seemed to overcome a negative effect of microgravity, whereas in the case of the centrifuge, the effects of vibration, if real, apparently added to the negative influence of hypergravity.

At this time, we have answered four questions. First, it seems clear that the HARV bioreactor effectively simulates microgravity. Second, this negatively impacts cardiac development, and (third) involves the alteration of fibronectin distribution, especially in basement membranes. Fourth, exposure to even small loads of hypergravity abolishes the development of spontaneous contractions. Our continuing analysis with immunoassay, image analysis, and electron microscopy should clarify and refine our conclusions.

All life has evolved at unit gravity. This has surely shaped biological systems, especially fundamental cellular activities and developmental processes. But it has been difficult to know how—to know what components of living matter are the targets for perception and adjustment to gravity, particularly at the cellular level. This is because we cannot easily manipulate gravity. However, a viable hypothesis has recently begun to accrue experimental support: that the complex network of macromolecules comprising the cellular cytoskeleton and the extracellular matrix, connected to one another through the cell surface via receptors and other membrane components and serving to mediate information transfer and dynamic adjustment for the cell, is sensitive to gravitational force (Spooner, 1994).

Fibronectin is an abundant extracellular matrix molecule that links the cell surface to the matrix. It is produced by cells and deposited at their surfaces where adhesions, migrations, and signaling to other cells will take place, events crucial to proper development of embryos. Formation of the embryonic heart is an experimentally accessible event where such adhesions, cell migrations, and signaling will occur. Our results suggest a sensitivity of heart myogenesis and fibronectin production to gravity. If the sensitivity to gravity and the importance in heart development of fibronectin can be determined, we will derive a more complete understanding of how gravity influences normal animal development and ability to reproduce.

FY97 Publications, Presentations, and Other Accomplishments:

Lwigale, P.Y. Effect of microgravity on heart development: Development of contractions, histiogenesis, and distribution of fibronectin. (Thesis) University of Northern Iowa (1997).

Norton, W., Spooner, B.S., and Wiens, D.J. "Effects of altered gravity on early chick heart development." (Poster) JOVE Annual Retreat, Pasadena, CA (1997).

Norton, W., Spooner, B.S., and Wiens, D.J. "Employment of a bioreactor vessel to simulate elements of microgravity during early chick heart development." (Poster) Microscopy and Microanalysis 55, 171-172, Microscopical Society of America Annual Meeting, Cleveland, OH (1997).

Markers for Assessing Vertebrate Development in Space

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Funding:

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Solicitation: 93-OLMSA-07
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Post-Doctoral Associates: 1

Task Description:

The long-range goal of the proposed research is to examine the effects of the space flight environment, including altered gravitational fields, on vertebrate development and cellular differentiation. Given the limited opportunities for flight experiments, ground-based studies are crucial as a means of evaluating both the aspects of development most likely to be affected in space as well as molecular and morphological markers of perturbed development. Furthermore, given the limited availability for experiments in mammals in flight, experiments are proposed to investigate, concomitantly, development of the fish *Medaka*.

A long range goal of our experiments was to identify sensitive markers for changes in cells in response to the unique environment of space flight. One of the promising candidates for sensing subtle changes in cellular homeostasis after exogenous stress are heat shock or stress proteins (hsp). We characterized the spatio-temporal pattern of expression of small heat shock protein hsp25 in the mammalian brain during acute exposure to heat shock or oxygen depletion. We made the surprising observation that the expression of hsp25 is specifically restricted to motor neurons within the murine central nervous system (Murashov et al., 1997). Immunocytochemistry was performed on paraformaldehyde-fixed free, floating sections of developing mouse brain at the above stages using an anti-hsp25 antibody (Stressgen) and the ABC horseradish peroxidase kit from Vector Laboratories. In the absence of stress, the expression of the hsp25 protein in adult mouse brain was restricted to motoneurons of the facial, trigeminal, hypoglossal, and ambiguous nuclei of the brain stem. Hsp25 was also detected in fibers of the facial and trigeminal nerve tracts, trigeminal sensory, and mesencephalic nuclei. To begin our analysis of altered environmental stimuli, we subjected 8-10 week old male mice to heat shock. The mice were lightly anesthetized with nembutal and placed on a warm pad (50°C). Core body temperature was monitored with a small rectal probe attached to a digital thermometer. Once body temperature reached 42°C, it was maintained for 15 minutes and then mice were allowed to recover for 2, 8, or 16 hours. The second altered environmental condition that was examined was hypoxia. The treatment consisted of one single exposure to oxygen depletion. Mice were placed in a special hypoxic chamber with an atmosphere of 5.2% oxygen, 5% CO₂, and the rest filled up with nitrogen. Mice were exposed to hypoxia for 2, 6, or 16 hours. After specified periods of time, animals were euthanized with CO₂ and tissues were processed for immunohistochemistry. The experiments showed that both heat shock and hypoxia treatment elicited expression of hsp25 in the adult mouse

brain. Significant increases in the levels of hsp25 were observed in the motoneurons of the facial, trigeminal, hypoglossal, and ambiguus nuclei of the brain stem, as well as in the trigeminal sensory, and mesencephalic nuclei and along the facial and trigeminal nerve tracts. The highly spatially restricted expression of hsp25 suggests that this protein may have a unique function in the orofacial motor nuclei system of the brain, controlling superficial and deep skeletal muscles of face and neck; the protein may also have a possible protective role in these motoneurons under conditions of stress and injury. Thus, our experiments showed that hsps can serve as sensitive markers of cell injury in different cell populations of mammalian brain after exogenous stress. In particular, hsp25 may serve as a unique and specific marker for cell injury in facial-trigeminal neuronal system.

This research seeks to understand the effect of space flight and microgravity on vertebrate development, in particular on the development and function of the central nervous system (CNS). The overall goal of the proposed study is to identify and evaluate sensitive molecular and cellular markers of vertebrate morphogenesis in order to assess the effects of the altered environment of space flight on embryonic and post-embryonic development. The hypothesis to be examined is that embryonic development (and neural development in particular) will be affected, potentially in a subtle but biologically significant manner, by exposure of the animals to the environment of space, and further, that this response will be different at different stages of embryonic and post-natal development of the animal. While our research does not seek to develop directly new therapeutics or protocols of alleviating symptoms of a disease or malady on Earth, it is extremely relevant to understanding the effects of altered environments on normal and abnormal human and animal development and in the etiology of pathological conditions that can occur, in particular, under stress. That is, this research will yield new understanding of basic biological processes, such as the regulation of gene expression in response to exogenous stress during early development and the molecular mechanisms involved in adaptation to microgravity. The success of developmental processes including fertilization, embryonic development, and maturation determines the ability of a species to survive in a certain environment. Space flight environment includes several hazards that potentially are able to affect developmental processes such as radiation, alterations in atmospheric pressure, prolonged toxic exposure, and microgravity. The impact of this research will be an increased awareness and comprehension of the importance of the effects of altered environments on life as we know it today. Space flight and space basic science provide a unique opportunity to evaluate the role of gravity in normal physiology and metabolism. The investigation of the influence of space flight environment on developmental processes is important in terms of evaluating possibilities of human survival in space.

FY97 Publications, Presentations, and Other Accomplishments:

Murashov, A.K. and Wolgemuth, D.J. "Expression of heat shock proteins in the gut after hypoxia and exogenous stress." *Cell Biology of Hypoxia*, Naval Medical Research Institute, p. 13 (1996).

Wolgemuth, D.J., Herrada, G., Kiss, S., Cannon, T., Forsstrom, C., Pranger, A.L., Weismann, W.P., Pearce, L., Whalon, B., and Phillips, C.R. "Vertebrate development in the environment of space: Models, mechanisms, and use of the *Medaka*." *ASGSB Bull.*, 10(2), 97-109 (1997).

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No Co-Is Assigned to this Task

Funding:

UPN/Project Identification: 199-99-17-03
 Initial Funding Date: 1994
 Students Funded Under Research: 0
 FY 1997 Funding: \$ 150,000

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 Expiration: 2000
 Post-Doctoral Associates: 4

Task Description:

The NASA Space Biology Research Associate Program (SBRP) has provided the opportunity to train scientists to conduct biological research in space and to continue relevant ground-based research since 1980. The research is conducted in laboratories that provide the necessary facilities and a suitable research environment. It is anticipated that these scientists will develop research careers in the newly evolving discipline of gravitational biology, a focused area of space biology. The field of gravitational biology is rapidly growing and its future will reflect the quality and training of its scientific personnel.

The Research Associate Program has completed the training of two post docs, Friedrich Behringer, Ph.D., and Andrew C. Ertl, Ph.D. Dr. Behringer is remaining in the laboratory of Terri Lomax, Ph.D. at Oregon State University, and Dr. Ertl is remaining at the laboratory of Dr. David Robertson at Vanderbilt University Medical Center. Dr. Ertl is also working as a Neurolab Coinvestigator and Payload Specialist microneurography Training Coordinator and Instructor.

Three new associates have entered the program since last year. Francis Pizza, Ph.D. is working on "Nitric oxide function in muscle response to modified loading" in the laboratory of Dr. James G. Tidball at UCLA. James Hurst, Ph.D., is working on "Effectiveness of growth hormone and high intensity exercise as a countermeasure to skeletal muscle atrophy" in the laboratory of Dr. Robert Fitts at Marquette University. Scott Gordon, Ph.D., is working on "Countermeasures to atrophy in unloaded skeletal muscle" in the laboratory of Dr. Frank Booth at the University of Texas Medical School at Houston.

The SBRA program is training people who will be the leading scientists in research in health and agriculture on Earth in addition to their space biology research.

FY97 Publications, Presentations, and Other Accomplishments:

Bangart, J.J., Widrick, J.J., and Fitts, R.H. "Effect of intermittent weight bearing on soleus fiber force-velocity power and force-pCa relationships." *J. Appl. Physiol.* (In Press).

Behringer, F.J. "Photobiology and genetics of the lazy-2 tomato mutant." Twelfth Annual Meeting of the American Society for Gravitational and Space Biology, Charlotte, NC (October 23 - 26, 1996).

Ertl, A.C. "LBNP exercise for long duration spaceflight." Aerospace Medical Association Annual Meeting, Chicago, IL (May 13, 1997).

Ertl, A.C., Jacob, G., Shannon, J., Robertson, R.M., and Robertson, D. "Evaluation of concomitant neurohumoral and plasma volume responses to the upright posture in normals." VII International Symposium of the Autonomic Nervous System, Montreal, Quebec, Canada (October 12, 1996).

Ertl, A.C., Watenpaugh, D.E., Fortney, S.M., Lee, S.M.C., Ballard, R.E., William, J.M., and Hargens, A.R. "Supine treadmill exercise with lower body negative pressure maintains upright exercise capacity following 14 days of bed rest." Twelfth Annual Meeting of the American Society of Gravitational and Space Biology, Charlotte, NC (October 23 - 26, 1996).

Widrick, J.J. and Fitts, R.H. "Peak force and maximal shortening velocity of soleus fibers after non-weight bearing and resistance exercise." *J. Appl. Physiol.*, 82, 189-195 (1997).

Widrick, J.J., Romatowski, J.G., Karhanek, M., and Fitts, R.H. "Contractile properties of rat, rhesus monkey, and human type I muscle fibers." *Am. J. Physiol.*, 272 (Regulatory Integrative Comp. Physiol. 41): R34-R42 (1997).

Plasmodesmata and the Control of Gravitropism

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Funding:

UPN/Project Identification: 199-40-57-22

Solicitation: 12-10&11-92/GB

Initial Funding Date: 1993

Expiration: 1998

Students Funded Under Research: 3

Post-Doctoral Associates: 1

FY 1997 Funding: \$65,000

Task Description:

Upward curvature of horizontal stems and coleoptiles occurs because of a lateral redistribution of auxin towards the lower side during the transduction and translocation phases of gravitropism. The evidence suggests that polar auxin transport undergoes a change so that the normal longitudinal transport becomes partly lateral. The following hypothesis is proposed to explain how this could occur. Polar auxin transport occurs primarily in starch-containing cells (statocytes). Sedimenting amyloplasts cause a localized concentration of Ca^{2+} at the base of the cell with two consequences: activation of IAA-efflux carriers at that wall, and closure of plasmodesmata (PDM) connecting the cell to the one below. The result is directional efflux of IAA with no back-diffusion through the PDM. When a tissue is reoriented horizontally, the reorientation of the amyloplasts to the lower longitudinal wall results in an increase of Ca^{2+} there, causing the IAA-efflux carriers along this wall to become active and the PDM connecting the statocyte to its lateral cells to close. The result is a polar transport of auxin laterally. Auxin then moves to the site of reaction, the elongating cells, where it causes excretion of protons into the apoplast. The lowered wall pH causes wall loosening and cell elongation. Because of the lateral movement of auxin, there will be an asymmetry of auxin across the organ, and thus a differential in apoplastic pH across the gravireacting organ.

The following experiments are proposed to test certain predictions that arise from this hypothesis. The first concerns the prediction that the location of statoliths in the statocytes will determine whether PDM between that cell and its neighbors are open or closed to small molecules. Two small fluorescent molecules will be microinjected into statocytes of *Avena* or maize coleoptiles. It is predicted that when the tissue is vertical, the dye will mainly move longitudinally rather than laterally. If the dye is injected into a non-statocyte, movement of dye will occur equally in all directions. The second prediction is that the cytoplasmic Ca^{2+} concentration will be higher at the bottom than the top of statocytes regardless of the orientation of these cells. This will be examined by confocal laser microscopy of starch-containing cells of *Avena* coleoptiles after injection with dextran containing the fluorescent dyes calgreen (Calcium Green) and rhodamine. Fluorescence ratio imaging will give the actual concentration at specific locations in the cell. It is predicted that the localized concentration of Ca^{2+} will be at the basal end of statocytes when the cells are vertical, but will be at the lower longitudinal wall when the cells are horizontal. The third prediction is that the apoplastic pH on the two sides of the organ will be different. This has never been tested directly because of the lack of a suitable technique to actually measure apoplastic pH. We propose to use the pH-sensitive fluorescent dye CL-NERF, ratioed against the

pH-insensitive dye Texas Red, to measure the apoplastic pH directly, and then determine whether there is a lower pH on the rapidly-growing lower side of an *Avena* coleoptile as compared with the slower-growing upper side.

Activities have been concentrated along three lines. The first is to continue the work on the movement of fluorescent dyes from cell-to-cell in the coleoptile, as influenced by the direction of the gravity vector. More information has been obtained about the coupling of *Avena* coleoptile cells, using injection of Lucifer Yellow or uptake of carboxyfluorescein (CF) diacetate, and then determination as to the amount of movement from cell to cell. It is becoming more apparent that only a small subset of cells are coupled, so as to allow small organic molecules such as CF to move from one cell to the next. In particular, the vascular parenchyma cells are well coupled. Gravity alters this coupling, in that it allows dye to move from the vascular parenchyma closest to the sieve cells to further parenchyma cells. But there is still no evidence that there is any coupling between the epidermal cells, which are believed to play a major role in the control of coleoptile elongation, and any of the cell layers beneath.

The second area of work has been on the confocal measurement of calcium, using fluorescent dyes. We had anticipated using Calcium Green/Texas Red conjugated to a dextran, and injected into the coleoptile cells. This dye combination has been used successfully in animal cells. But in our hands, it fails to work in plant cells. We have since learned that others have run into the same problem. As a result, we are now examining the possibility of using a UV-confocal and Indo-1 dye to measure intracellular calcium.

The third, and most successful avenue of research concerns the use of CL-NERF and Texas Red-Dextran to measure apoplastic pH. Again we are using *Avena* coleoptiles for this. It has taken a considerable period to learn how to infiltrate the dyes into the walls without getting them taken up into the cells themselves. We then had to learn how to calibrate the pH *in situ*, using these dyes. This has now been successfully done. We have found that the pH of the wall is about 4.8-5.0 in the absence of auxin or fusicoccin. If fusicoccin is added, there is no change in the apoplastic pH for about 3 minutes, but then there is a decrease in apoplastic pH of about 0.5 pH units within a few minutes. We are currently repeating these measurement using auxin as the agent to alter the wall pH. These are, we believe, the first reliable measurements of the pH of the cell wall solution. We are now approaching the time when we can do the measurements of wall pH on the two sides of the gravitropically-bending coleoptile, and test our hypothesis that there will be a differential in wall pH prior to the start of the bending.

The Earth benefits of this research fall into two areas: benefits to an understanding of basic biological processes and benefits to agriculture. Plants consist of a multitude of cells, fixed in position by their walls, and interconnected by plasmodesmata into a "symplast." The plasmodesmata are believed to permit small molecules (ones smaller than about 800 Da) to pass freely from cell to cell. This would include sugars, amino acids, ions, and of course plant hormones. This raises some important questions. How can cells end up differentiating into different cell types when they are contiguous and if they are subjected to the same chemical environment? How could gradients of morphogenetic factors exist if the cells are really freely interconnected? Is there any control of the movement of small molecules through the plasmodesmata? The research being conducted under this task is some of the first work on the cell-to-conductance in growing and developing tissues. Until now, most research on plasmodesmata has focused on one of two systems—mature leaf mesophyll cells, and phloem companion and parenchyma cells. Neither of these is a tissue in which morphogenetic gradients is expected to play an important role. As a result, our knowledge about the plasmodesmal conductance of developing tissues is limited. The research conducted here indicates that the conductance is far more limited than had been realized. It indicates that developing cells may exert real control over the ability of hormones to move from cell to cell. It is the start of what should prove to be an important area of plant research.

Plasmodesmal conductance is an important topic in agriculture for several reasons. First, the spread of viruses in plants from cell to cell occurs through the plasmodesmata. Each virus codes for a movement protein which causes a huge increase in the size exclusion limit of the plasmodesmata and carries the viral nucleic acid through the plasmodesmata. But how do these movement proteins exert their effect? Until we know far more about the control of the plasmodesmal conductance we cannot answer that, or devise effective ways of preventing the viral nucleic acid-movement protein from actually moving. A second important question is how the growing

meristems of root and shoot are supplied with the nutrients needed for growth. It has been postulated that in the root, sugars unloaded via plasmodesmata from the sieve tubes into parenchyma cells then move to the meristem via the plasmodesmata. But if the plasmodesmata are really closed, as my research suggests, alternative movement pathways must occur. The results of this work may point the way to future research which will provide answers to these questions.

FY97 Publications, Presentations, and Other Accomplishments:

Cleland, R.E. "General responses on graviresponses." *Planta*, 203, S170-173 (1997).

Plant Gravitropisms and the Role of Expansins

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Funding:

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Students Funded Under Research: 1

Post-Doctoral Associates: 1

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The general goal of this research is to elucidate the cellular and molecular mechanisms by which gravity alters the growth of plants. Plant gravitropisms involve an asymmetry in the rate of cell enlargement and wall expansion on the two sides of the bending organ. However, relatively little is known about the detailed molecular mechanisms by which asymmetric cell wall expansion is established and controlled during gravitropism. Published work indicates that an asymmetry in "acid growth" may be partly responsible for the growth changes. The experiments proposed here build on our recent discovery of the proteins ("expansins") that mediate this acid-growth mechanism. We propose experiments to analyze expansin gene expression, expansin protein activity, and wall sensitivity to expansin action during gravitropism of cucumber and *Arabidopsis* seedlings. We will use expansin mutants and transgenic plants with altered expansin levels to characterize the sensitivity of gravitropic bending to expansin content. Furthermore, we will use PCR to clone and sequence expansins from divergent taxa as a means of discovering conserved (functional) domains of the protein and as a prelude to future work with these species. The results should advance our understanding of the molecular machinery that controls plant cell expansion in general and how gravity (via the gravitropism transduction pathway) interacts with this machinery to modulate plant cell expansion.

1) The ability of cucumber hypocotyl cell walls to extend *in vitro* was found to depend on the activity of expansin, the "susceptibility" of the wall to expansin action, and the wall pH. In the cucumber hypocotyl, we find a good correlation between endogenous growth, wall creep *in vitro*, and expansin transcript abundance. This is consistent with the hypothesis that expansin gene expression is an important determinant of cell wall extensibility. In addition, there is also a close correlation between endogenous growth rate along the hypocotyl and the susceptibility of the wall to respond to expansins. This indicates that other factors, besides expansin expression, contribute to wall extensibility. In the growing cucumber hypocotyl, expansin genes EXP1 and EXP2 are differentially regulated by light, auxin, and gibberellin. This suggests that there are important functional differences in the two expansins, and that EXP2 is the more promising candidate for involvement in gravitropism (because EXP2 is upregulated by auxin).

2) Wall pH is likely to modify the wall extensibility in multiple ways. First, there is a purely physical effect on the wall pectins and other charged polymers in the wall. Second, pH affects wall enzyme activity: expansins, endoglucanases, glycosyltransferases, and pectin methyl esterases have different pH optima, and their effects on the wall are likely to interact to increase wall extensibility at low pH and reduce wall extensibility at neutral pH.

3) We are currently in the early stages of characterizing *Arabidopsis* plants with altered expansin gene expression. This is complicated by the fact that *Arabidopsis* has at least 13 different expressed expansin genes. Efforts are underway to identify the expression patterns of these genes by *in situ* hybridization, specifically to identify which expansin genes might be involved in the gravitropism of hypocotyls, roots, and inflorescences.

4) We have identified expansin genes expressed in tobacco BY2 cell suspension cultures and generated transgenic cell lines with expansin antisense and sense overexpression. The intent is to analyze the alteration in growth and wall properties in these cell lines with altered expansin expression.

This research should provide insights into the basic cellular and molecular mechanisms by which plants regulate their growth and how gravity interacts with plant growth control processes. Such knowledge will provide the background for future attempts to engineer plant growth to emphasize favorable traits, such as fast growth rate, altered wall structure, and ability to withstand environmental stresses.

FY97 Publications, Presentations, and Other Accomplishments:

Cosgrove, D.J. "Assembly and enlargement of the primary cell wall in plants." *Annu. Rev. Cell Dev. Biol.*, 13, 171-201 (1997).

Cosgrove, D.J. "Cellular mechanisms underlying growth asymmetry during stem gravitropism." *Planta*, 203 (Supplement), 130-135 (1997).

Cosgrove, D.J. "Plant cell enlargement and the action of expansins." *BioEssays*, 18, 533-540 (1996).

Cosgrove, D.J. "Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement." *Plant Cell*, 9(7), 1031-1041 (1997).

Regenerating Protoplast as a Single-Cell Model System for Studying the Gravitropism in Plants

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Funding:

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Expiration: 2001

Students Funded Under Research: 0

Post-Doctoral Associates: 0

FY 1997 Funding: \$131,396

Task Description:

Plants sense and developmentally respond to gravity using cellular mechanism which are not well understood. For humans to establish an effective presence in space, more must be learned about how plants respond to gravity, and how the microgravity environment affects their growth processes. Recently, we published data (*Plant Physiol.* 110:425-430, 1996) showing that a brief centrifugal force predisposes regenerating protoplasts to elongate in a non-random fashion. We now find that gravity can also affect the growth direction in these single cells of tobacco. The finding that gravity induces a non-random growth pattern demonstrates that a single cell, from a higher plant, has all the necessary elements to morphogenetically respond to gravity. These cells are easy to work with and study individually, making them an attractive model to use for gravity studies. After fully characterizing how these cells behave towards gravity, we will explore experimentally the concept that a number of cellular elements are involved in gravitropism and function in a coordinated manner to affect directional growth. Perturbations of the cytoskeleton will test whether this structure alters the cell's ability to perceive and respond to gravity and how the endomembrane system is concomitantly affected. The role that dense organelles play in gravity perception and concomitant growth will also be investigated. How cytoplasmic density affects gravity perception will be explored by increasing the density of this cellular compartment and by introducing ferromagnetic particles which can then be directionally moved in a magnetic field. The biophysical role that transvacuolar strands play in transducing resultant gravitational biophysical force will be explored using laser tweezers and laser ablation techniques on a horizontally mounted microscope. We will also propose a microgravity experiment that could be done in the mid-deck locker of the Shuttle by 1998.

Plants are currently thought to perceive gravity using highly specialized cells that contain heavy plastids. Once gravity is sensed, these cells are believed to relay this information to other plant cells. We have data to indicate that less specialized cells may sense and respond to gravity as well. If this is correct, then the basic gravireception/response mechanisms are more prevalent than previous thought and these non-specialized cells will predictably grow differently in space as they will lack a gravitational cue.

Agriculture depends directly, or indirectly upon the optimal growth of plants. To make informed decisions requires an intimate knowledge of plant growth processes. Plants use gravity as a major developmental cue to direct shoots to grow upwards and roots to grow downward. How this is done is unclear. The present research program will provide additional insight into how plants sense and respond to this important developmental cue.

The addition of this information to our basic knowledge will allow us to make more intelligent decisions about how to more effectively grow agronomically important crops.

This project did not begin until late FY 1997 and is, therefore, in its early stages.

Cellular Specificity in Arabidopsis Root Gravitropism

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Funding:

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Solicitation: 91-OSSA-15

Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$97,043

Task Description:

There is uncertainty concerning which cells in plant roots detect gravity and which cells carry out the motor response leading to reorientation. There is also uncertainty concerning the extent to which the plant growth hormone, auxin, is the key mediator of the gravitropic motor response. It is important to resolve these questions precisely if we are to understand the gravitropic response in plants. We have developed methodology that allows us to make precise measurements of angle of orientation of root subsections and simultaneously analyze localized growth rate distribution patterns. Our preliminary studies indicate that there are at least two motor response regions in roots. We hypothesize that there are also at least two gravity detecting zones — cells in the cap where starch-containing amyloplasts sediment, and a second zone within the root proper. The main thrust of this research is to characterize the interaction of these potential multiple detectors/motors in root gravitropism. In order to do this, we will modify our current equipment to allow feedback between our growth/angle measuring equipment and a new seedling orientation device. Using this new methodology to study both normal and starchless (missing the main gravi-detecting machinery) mutants of *Arabidopsis* and tobacco, we will determine the major zones of gravity sensing and compare these zones to recently discovered multiple motor regions. We expect these studies to lead to a firm understanding of gravity sensing zones in the root, possibly revealing, for the first time, gravisensing external to the root cap. We also expect these studies to determine whether some cells in the root possess both gravity detecting and motor response capabilities. The emphasis of research during the first year is to use existing technology to compare the location of the motor cells in wild type and starch-deficient mutants of *Arabidopsis* and tobacco, to construct the new closed loop feedback system for control of seedling orientation, and to complete software required for data analysis. We would also plan to begin the comparison of zones of gravisensing in wild type and starchless mutants of *Arabidopsis* and tobacco during the first year. During the second year, we would complete the study of localization of zones of gravisensing and begin a study of the role of the extracellular matrix (as an alternative to the amyloplast sedimentation hypothesis) in gravisensing. The emphasis during the third year would be on the analysis of gravitropism in auxin overproducer transgenics and in auxin/gravitropism response mutants of *Arabidopsis*.

During the past year we completed the software required for feedback control of root orientation. This new software will be used in conjunction with our video digitizer system for analysis of root growth and gravitropism. The new system allows us to control the angle of orientation of a defined subsection of the small roots of *Arabidopsis*. This system will be used in the coming year to map gravi-sensing activity along the root

tip. In addition to this technical advance, we have made a major improvement in the resolution of our surface expansion analysis capability with the tiny roots of *Arabidopsis*. In particular, we have developed a method for microbead application to the root surface so that we can apply markers throughout the root surface. This has been coupled with a modification of software that will soon allow us to determine localized surface expansion patterns in roots of *Arabidopsis* as we have done in the past for roots of maize. This analysis is a challenge for roots of *Arabidopsis* both because of their small size and because the roots tend to exhibit a complex pattern of circumnutation or waving during growth. We have begun to analyze which populations of cells drive circumnutation and which populations mediate responses to tactile stimulation and gravistimulation. Responses to gravistimulation occur in cells closer to the root tip while circumnutation and responses to touch occur more basally. Earlier in these studies we determined that growth in the more basal regions is controlled by auxin while growth closer to the tip is less dependent on auxin. In an effort to determine which factors control cell expansion near the root tip (i.e., in the distal elongation zone, DEZ), we have examined the influence of ion channel blockers on growth responses in that region. We find that anion channel blockers such as NPPB strongly inhibit the gravitropic growth response while having little effect on vertical growth. Interestingly, the effectiveness of NPPB seems to be highly dependent on localized site of application (upper DEZ is much more sensitive) suggesting asymmetry of anion channel activation during the gravitropic response of the DEZ.

This research focuses on an analysis of the cellular mechanisms of plant responses to gravity. The research involves the development of new technology for precise measurements of plant growth and orientation. It is expected that this research will lead to a more complete understanding of how plants sense and respond to gravity. Because it is likely that plant responses to gravity share many features in common with responses to other environmental factors (light, temperature, touch), it is expected that advancing our understanding of plant response mechanisms will lead to improvements in optimizing plant growth under a variety of conditions. It is also likely that these advances will enhance our success of growing plants in novel (e.g., space) environments. In addition to these benefits, there is a more general benefit to the research community to be realized from our development of computerized growth analysis. We have begun developing a plant growth imaging web site devoted to automated analysis of plant growth and standardization of plant growth experimental conditions. This is anticipated to provide a means for greatly accelerating the progress of the research community in the understanding of plant growth.

FY97 Publications, Presentations, and Other Accomplishments:

Evans, M. "Molecular and physiological characterization of the distal elongation zone in roots." Joint Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997).

Evans, M. "The NASA/NSF Joint Program in Plant Biology. Activities of the Network for Research on Plant Sensory Systems." Joint Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997).

Evans, M. "Thigmotropism, gravitropism and circumnutation in *Arabidopsis* roots: High resolution analysis of the role of the distal elongation zone." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (September, 1997).

Evans, M.L. and Ishikawa, H. "Cellular specificity of the gravitropic motor response in roots." *Planta*, 203, S115-122 (1997).

Evans, M.L. and Ishikawa, H. "Computer based imaging and analysis of root gravitropism." *ASGSB Bull.*, 10, 65-73 (1997).

Ishikawa, H., and Evans, M.L. "Novel software for analysis of root gravitropism: Comparative response patterns of *Arabidopsis* wild type and *axr1* seedlings." *Plant, Cell & Environ.*, 20, 919-928 (1997).

Ishikawa, H. and Evans, M.L. "Use of computerized video imaging technology for analysis of differential growth patterns during root gravitropism." First Annual Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997).

Loomis, J., Liu, X., Ding, Z., Fujimura, K., Ishikawa, H., and Evans, M.L. "Visualization of plant growth." Proceedings of the Conference on Visualization, Institute of Electrical & Electronic Engineers (In Press).

Wolverton, C., Evans, M., Ishikawa, H., and Spalding, E. "Suppression of *Arabidopsis* root gravitropism by the anion channel inhibitor, NPPB [5-nitro-2-(3-phenylpropylamino)-benzoic acid]." *Plant Physiology* 114: Suppl. p 262. Annual meeting, American Society of Plant Physiologists, Vancouver, British Columbia (1997).

Young, L. and Evans, M.L. "Patterns of auxin and abscisic acid movement in the tips of gravistimulated primary roots of maize." *Plant Growth Regul.*, 20, 253-258 (1996).

Young, L., Evans, M.L., Ishikawa, H., Wolverton, C., Simmons, C., and Söll, D. "The gravitropic response of primary roots of the *rgl1* mutant of *Arabidopsis thaliana*" in "Plants In Space Biology." Edited by: Suge, H. and Takahashi, H. Tohoku University Press, pp 73-81 (1996).

The Use of Arabidopsis Transposon Mutants in the Study of Gravitropism

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Task Description:

The objective of the experimental plan described here is to use 3 newly identified *Arabidopsis* insertion mutants to study different aspects of plant interactions with a gravitational field. We have identified 3 insertion mutations in which transposons carrying promoterless reporter genes have transposed into genes that may either be directly involved in graviperception or are expressed exclusively in tissues central to graviperception in roots. Two of the insertion mutations are in genes expressed only in root cap cells, as judged by the localized expression of the transposon-borne promoterless beta-glucuronidase (GUS). In one of the mutants, the gene is expressed throughout the root cap, while in the other, expression of the reporter gene is confined to the central columellar cells. A third recently identified mutant resembles the diageotropica (dgt) mutant of tomato. All of these mutants will initially be characterized physiologically to determine whether they show altered root gravitropism. The genes will be cloned, sequenced, and examined for homologies with genes of known function. The coding sequences of the genes whose disruption affects root gravitropism will be expressed ectopically from promoters other than their own. Such experiments will address the question of whether mutations that affect root graviperception do so by virtue of what they encode or where they are expressed. In a different type of promoter-exchange experiment, the coding sequence of the tagged genes that are expressed in the root cap will be used to express a temperature-sensitive diphtheria toxin A subunit gene, a cell autonomous lethal toxin. Transgenic plants carrying the toxin gene constructs will be made and used to study the effects of temperature-shift experiments on root graviperception. If root caps (and, in particular, the columellar cells) are, as is generally believed, the site of graviperception, these experiments will permit us to study the effects of ablating specific groups of cells transiently throughout the plant, something that has not been possible hitherto. Finally, analysis of the dgt-like mutant of *Arabidopsis*, together with the ability to overexpress and study the localization of the gene product, may well provide insights into the mechanism by which the signal is transmitted and/or the root responds to the signal.

We have carried out preliminary work with all three of the transposon insertion mutations described in our proposal. We have analyzed a gene detected by a GUS-transposon insertion exhibiting GUS expression only in root cap cells (line 102). Gene-specific probes homologous to the flanking sequences were used to identify an *Arabidopsis* EST and to clone the gene. The transposon inserted just upstream from the start codon. The gene comprises 8 exons and 7 introns and encodes a 415-amino acid ORF with no significant homology with any known gene in the GenBank database. Expression of the gene, as judged by expression of the GUS gene on the inserted transposon, commences several hours after germination and is seen in root cap cells of both primary and

lateral roots. To determine whether expression of the gene is abolished by the transposon insertion, RNA blots containing polyA⁺ RNA isolated from roots of wild-type and line 102 plants were probed with a labeled EST cDNA fragment. The probe hybridized to a 1.4 kb and an 0.9-kb mRNA in roots of both wild-type and 102 plants. However, the intensity of the two bands was approximately equal in RNA from wild-type plants, while the intensity of the 0.9-kb band was stronger than that of the 1.4-kb band in RNA from 102 plants. This observation suggests that expression of the gene has not been entirely disrupted by the *Ds* insertion. This may be due to weak promoter activity associated with the 3' region of *Ds* element recently observed in our laboratory.

To determine the function of this gene, transgenic *Arabidopsis* lines which express antisense RNA of the EST cDNA were created. The cauliflower mosaic virus 35S promoter (CaMV 35S) was fused to the EST cDNA in an antisense direction, so that the CaMV 35S is expected to drive expression of antisense RNA from the cDNA in the transgenic plants. T-DNA containing the antisense construct was used to transform Nossen ecotype *Arabidopsis* plants. Preliminary characterization of T2 plants shows that transgenic lines exhibit an exaggerated right-slanting root-growth phenotype on the surface of 1.5% agar medium tilted 30° from the vertical. Roots of the transgenic lines slant more to the right than wild-type *Arabidopsis* Nossen roots. Roots in seedlings of wild-type *Arabidopsis*, the Wassilewskija, Landsberg *erecta* and Nossen ecotypes often grow aslant on vertical agar surfaces. Slanted growth of roots always occurs to the right of the gravity vector when the roots is viewed through the agar surface, and is not observed in the Columbia ecotype. The degree of exaggeration of right-slanting root-growth phenotype on agar surfaces varies among the transgenic lines. Because the phenotype was seen in independent transgenic lines, it is unlikely that the phenotype was caused by gene disruption due to the T-DNA insertion. Rather, it appears to be due to the presence of the antisense construct.

It has been suggested that native directional growth bias exists in *Arabidopsis* roots. The repression of expression of this gene by over-expression of antisense RNA may be disturbing a system involved in the autotropism. It is conceivable that tropism induced by environmental stimuli may be accomplished through regulating the autotropism. Further characterization of the phenotype detected in the transgenic lines is underway. It would be very interesting to see the phenotype in transgenic plants in which the antisense RNA is expressed under a root-cap specific promoter and we are preparation such a construct.

To examine the consequences of root cap ablation throughout the plant, the promoter region of the gene was identified and used to drive expression of the diphtheria toxin A chain (DT-A) gene. The 1.4-kb upstream sequence was found to be sufficient to impose root cap-specific expression on a GUS gene. This promoter fragment was then used to drive expression of the DT-A gene, which inhibits protein synthesis by ribosylating the EF2 translation initiation. Transgenic lines expressing the DT-A gene have been obtained and preliminary studies show that all but 2-3 layers of root cap cells are eliminated as a consequence of toxin gene expression. The radial organization of the roots in the transgenic lines remains normal, although their root meristematic activities are lower than those characteristic of normal roots. Moreover, vacuolization and development of the vascular system and root hairs occur much closer to the root tip than in wild-type roots. Roots expressing the DT-A gene are shorter than those of wild type, lack the ability to sense gravity and may also be affected in touch perception. Preliminary studies suggest that growth of the primary root is much more extensively affected than the growth of adventitious and lateral roots, although root cap ablation is similar in both tissues. This observation suggests a different signaling role for the primary root cap than for those of the lateral roots.

The effects of plant hormones on root meristematic activity were tested by adding 2,4-dichlorophenoxyacetic acid, indole-3-acetic acid, 6-benzylaminopurine, gibberellic acid, and 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, to the medium on which plants were grown. None of the growth regulators rescue the short-root phenotype of the transgenic lines, suggesting that decreased meristematic activity is not attributable to a shortage of any of these growth regulators.

Preliminary analysis of a line originally identified by expression of the transposon-borne GUS gene only in columellar cells has revealed that the gene is also expressed in the quiescent center and the cortex/endodermal initials. Developmental studies show that gene expression is confined to the quiescent center immediately after imbibition and later becomes detectable in the cortex/endodermal initial cells and the columella cells. During

embryogenesis, gene expression is first observable in the region corresponding to the central cells at about the heart-stage of embryonic development and later becomes detectable in the columellar cells. During the early seed development, the gene is also expressed in the region corresponding to the chalazal nucellus, the basal part of a plant ovule where the nucellus is fused to the surrounding integument and to which the funiculus is usually attached. The region containing the insertion site has been cloned and is presently being sequenced.

Finally, we have begun studies on a mutant whose phenotype resembles that of the *dgt* mutant of tomato in the sense that both shoots and roots exhibit lateral growth. Our initial efforts have been focused on the identification of a revertant in order to establish that the mutant gene is transposon tagged. The mutant has reduced fertility because of aberrant flowers and therefore gives a low seed yield. We have not yet succeeded in identifying what we believe to be a true revertant, since the small number of wild-type plants obtained from mutants homozygous for the mutation and containing the *Ac* transposase gene have no evidence of a transposon "footprint" at the known insertion site. We believe it most likely that these arose by cross-pollination from wild-type plants. We are therefore taking a different approach to determining the linkage between the insertion and the mutation by analyzing mutant homozygotes lacking the transposase gene for recombinant chromosomes lacking the transposon. We have also begun to characterize this mutant morphologically and physiologically.

The benefits of this work derive from the insight they will provide into how plants grow, what functions are served by root caps and how plants respond to gravity. We have made the novel observation that plants totally lacking the ability to sense gravitropism through their roots can survive in the laboratory, even when growing in soil. Our next challenge is to determine how well they survive in competition with plants under normal field conditions and under various conditions of environmental stress.

FY97 Publications, Presentations, and Other Accomplishments:

Tsugeki, R., Olson, M., and Fedoroff, N. "Transposon insertional mutagenesis and the study of plant root development." Proc. Root Biology Symposium (In Press).

Transduction of the Gravity Signal in Roots of Corn

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Task Description:

The long-term objective of our research is to elucidate the molecular mechanisms of the transduction of gravity in roots. Recent evidence indicates that the transduction of gravity and light stimuli in roots involves second-messenger-dependent protein phosphorylation (Raghothama et al., 1987; McFadden and Poovaiah, 1988) and regulation of transcription (Feldman et al., 1988). To begin elucidating the molecular mechanisms of these transduction systems, a maize root cDNA (90.7) encoding a protein homologous to the conserved catalytic domain of second messenger-dependent protein kinases was isolated, cloned, sequenced, and expressed in *E. coli* (Biermann et al., 1990). Because calcium and calmodulin are hypothesized to be involved in root gravitropism we next directed our attention to isolating calcium/calmodulin-regulated protein kinases. To date we have isolated and sequenced two of these kinases. In order to establish a role for these kinases we have transformed both maize and tobacco plants and put these kinase genes under constitutive promoters. In addition we have carried out so-called "knock-out" experiments to eliminate the functioning of these genes in maize. Currently we are characterizing the phenotypes from these two approaches.

Since our previous work suggested that root gravitropism is a calcium-regulated response, and that both calmodulin and calcium/calmodulin-dependent kinases participate in this response, we have begun to characterize these kinases using a molecular approach. A maize root cap library was screened for calcium/calmodulin-dependent kinases and a genomic sequence has been obtained for two such calcium/calmodulin-dependent kinase homologs (*MCK1*, *MCK2*). These kinase homologs are expressed in root caps, the site of perception for both light and gravity. *MCK1* consists of 7265 base pairs and contains 11 exons and 10 introns. *MCK1* is expressed constitutively, in both light and dark, and therefore it is unlikely that the light directly affects *MCK1* expression, though the activity of the protein may be affected by light. In cultivars showing light-regulated gravitropism, we hypothesize that *MCK1*, or a homolog, functions in establishing the auxin asymmetry necessary for orthogravitropism.

We also attempted to eliminate (knock-out) calcium-calmodulin activity using maize plants in which genes for this homolog are interrupted or are otherwise non-functional. However, our efforts did not produce a distinct phenotype, leading us to conclude that calcium-calmodulin-dependent kinases are likely family consisting of at least three distinct genes and suggests that if this gene, or a homolog, is involved in gravity signal transduction, that there may be several redundant pathways.

Future work will be directed towards characterizing the protein products of these genes, including producing antibodies to purify the native, endogenous proteins.

The work seeks to identify the steps/processes involved in the transduction of a gravity signal in plants. Identification of players in this transduction scheme is the main focus of the work. By concentrating on kinases, an hypothesized key player, we are in a strong position to dissect steps of the gravity signal transduction pathway. These steps will likely be common to all plants and hence this work will contribute to understanding gravity signal transduction within the plant kingdom.

FY97 Publications, Presentations, and Other Accomplishments:

Feldman, L.J. "Evolution of the gravity signal transduction pathway in plants." *Astrobiology Workshop*, Ames Research Center (1996).

Feldman, L.J. "Gravitropism in roots." *Gordon Conference: Gravitational Effects on Living Systems* (1996).

Feldman, L.J. "Light-regulated gravitropism: A role for protein kinases." *International Workshop on Plant Biology in Space*. Bonn, Germany (1996).

Feldman, L.J. and Lu, Y-T. "Light-regulated root gravitropism: A role for and characterization of a calcium/calmodulin-dependent protein kinase homolog." *ASGSB Bull.*, 10, 72 (1996).

Lu, Y-T. and L.J. Feldman "Characterization of a calcium/calmodulin-dependent protein kinase homolog from maize roots showing light-regulated gravitropism." *Planta*, 199, 18-24 (1996).

Mechanism of Phytochrome Regulation of Shoot Gravitropism in Arabidopsis

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Task Description:

Plants have highly sensitive and selective mechanisms for sensing and responding to the Earth's gravitational field. These gravity response systems can be modulated by other signals in the environment such as light. Recent work in our laboratory has demonstrated that *Arabidopsis thaliana* seedlings provide a useful model system for investigating interactions between gravitropism and the phytochrome photosensory system. Specifically, dark-grown seedlings exhibit strong negative gravitropism, but red light irradiation severely attenuates negative gravitropism of the hypocotyls. The light-stable phytochrome B was found to be the phytochrome that mediates this response.

The overall objectives of this proposal are to determine at the cellular and molecular level how gravity responses in plants are modulated by light through the action of phytochrome using *Arabidopsis* as a model system. Specific goals of the proposed research are to conduct a detailed characterization of the interactions between different phytochromes and the gravitropic response system, and to conduct an analysis of the molecular components of the interaction. This will involve the use of wild-type plants, mutant strains that carry specific phytochrome mutations, and transgenic lines that contain engineered phytochrome genes to investigate phytochrome regulation of gravitropism in hypocotyls, roots, and flowering stems before and after reorientation. New mutants and second site revertants of specific mutations will be generated in order to identify portions of the gravity response system that interact with phytochrome. The proposed research is expected to provide a molecular handle for investigating signal transduction events that guide gravitropic response. As such, this research is relevant to the NASA Space Biology Program in that it will help to elucidate the mechanisms for perceiving and responding to gravity.

We have characterized a mutation (*hgr1*) that is responsible for an abnormal gravitropic response that was previously attributed to a lesion in the phytochrome B gene. The *hgr1* mutation has been mapped to a position on chromosome 1 approximately 20 cM between the physical markers AthGENEA and AthATPase. The phenotype and map position of this gene are very similar to a recently described mutant, *arg1*, and we expect that *hgr1* is allelic to *arg1*. We have obtained seeds of *arg1* and have made crosses to conduct allelism tests. The hypocotyls and roots of *hgr1* plants display agravitropic (random orientation) growth in darkness. After 90°

reorientation treatments, *hgr1* hypocotyls and roots develop minimal gravicurvature compared to wild type. Etiolated seedlings exposed to unilateral blue light showed stronger root phototropism compared to wild type roots. That *hgr1* develops phototropic curvature indicates that the mutation is specific to gravitropic signal transduction. The increased phototropic curvature is consistent with the idea that phototropism is normally limited by competition with gravitropism. In contrast to the lack of gravitropic response in hypocotyls, inflorescence stems display gravitropism. Upon reorientation by 90°, the inflorescence of *hgr1* plants show initiation of gravicurvature with approximately the same kinetics as wild type. However, time-lapse analysis of inflorescence growth reveals that after the initiation of gravicurvature, the inflorescence undergoes exaggerated nutational movements and it takes several hours before the plants settle into a vertical growth orientation. Time-lapse studies indicate that graviperception is functional in *hgr1* but that the response is abnormal, suggesting that *hgr1* functions downstream in signal transduction. We also found that *hgr1* shows wild-type sensitivity to IAA and NPA with respect to inhibition of hypocotyl and root elongation.

The time-lapse system we have developed for monitoring gravitropism is extremely simple and inexpensive. During the past year, we adapted the system for teaching students about plant movements, including phototropism. We presented a poster and demonstration of the system at the annual meeting of the American Society of Plant Physiologists. In addition, we developed a web page (sunflower.bio.indiana.edu/~rhangart) that provides detailed plans for using the imaging system for monitoring plant movements. Detailed plans for making useful modifications to the camera (e.g., infrared sensitivity) are also provided in addition to a number of time-lapse movies that can be viewed in web browsers. The web pages and the imaging system are already being used by teachers and researchers across the country.

We have completed the characterization 16 auxin- and ethylene-related mutants and have identified three that have altered gravitropic behavior and that do not show the red-light-induced changes in gravitropism that we have previously demonstrated to be typical of *Arabidopsis* hypocotyls. These three mutants are *axr1-3*, *axr5*, and *tir5*. The *axr1-3* and *axr5* were originally isolated as auxin response mutants and *tir5* was isolated as an auxin transport inhibitor resistant mutant. The *axr5* and *tir5* completely lack phyA- and phyB-dependent effects on their gravity response but other phyA and phyB responses are unchanged. The *axr1-3* mutant shows partial impairment of the phytochrome effect on gravitropism but is normal in other phytochrome responses. Because of its potential involvement in auxin transport, we are focusing our attention on *tir5*.

We have also been conducting physiological studies of the interaction between phytochrome and the gravitropic responses of leaves in *Arabidopsis*. We have found that leaf angle is controlled by red:far-red ratios. For example, leaves are held at higher angles at low red:far-red ratios in comparison to plants exposed to high red:far-red ratios. Leaf angle is also partly controlled by a circadian regulator. Analysis of specific phytochrome mutants has shown that phytochromes control the changes in leaf angle. Initial studies indicated that the phytochrome-dependent control of leaf angle is partly due to an effect of phytochrome on the gravitropic response of the leaves. However, this has been difficult to document with quantitative data. Our current working hypothesis is that in nature, the phytochrome changes in leaf arrangement may increase the competitive ability of plants growing in dense stands. Our findings also suggest that light quality within a plant canopy may determine the angle of branching as well as leaf angle and, thus, could be an important regulator of overall plant morphology. We are attempting to isolate mutants that display altered leaf orientation under various light conditions for investigating the gravitropic responses of lateral organs and their importance to plant development.

We have analyzed a series of phytochrome null mutants as well as transgenic plants overexpressing wild-type and mutant forms of phytochromes A and B. We have determined that the phytochromes modulate phototropism as well as gravitropism. In addition, we have found that in plants overexpressing phyA and phyB, the these phytochromes interact in certain phytochrome responses in a fashion that suggests that they may function as heterodimers. With some phytochrome responses, phyA and phyB appear to interact at a common site when the light signal is transduced. Analyses of the individual phytochrome mutants and transgenic plants suggest that phyA may be primarily responsible for increasing blue light sensitivity while phyB may be more important for altering the gravitropic response.

Our investigations on the interactions of gravity responses and the different photosensory systems in *Arabidopsis* are providing insights into the nature of the complex network of sensory response systems that regulate plant development. The gravitropic response system is clearly a central component of this environmental sensory network.

Plant morphology is an important agronomic trait that affects plant productivity. For example, branching patterns can affect overall photosynthetic capacity of a plant and, thus, alter yield. In addition, the angle of branch growth can affect spacing of plants and impact planting density. Because gravitropism affects these and many other aspects of plant growth, understanding how gravity helps shape a plant into its final form is not only of fundamental importance for understanding plant growth and development, but may have important agronomic implications. Our discovery that different photosensory systems modulate the gravitropic responses in aerial parts of plants suggest that it may be possible to engineer plants that will display growth habits that are suitable to a wider range of growth practices than are currently available. For example, since genes for the various phytochromes have been cloned, it is possible to change the levels of specific phytochromes in specific organs of a transgenic plant. By understanding how the different phytochromes affect gravitropism and thus affect branch angles, it should be possible to use the information from our research to improve yield potential for some crops. For example, by modifying the ratio of phyA and phyB in branches, it may be possible to construct a plant that will have more upright branches and allow closer planting while maintaining a high photosynthetic capacity and possibly resulting in higher yields.

FY97 Publications, Presentations, and Other Accomplishments:

Goud, K.V. and Hangarter, R.P. "Exploring functional interactions between phytochromes." 8th International Conference on *Arabidopsis* Research, Madison, WI (1997).

Hangarter, R. "Gravity and plant form." *Plant, Cell & Environ.*, 20, 796-800 (1997).

Jenesen, C.J.P. and Hangarter, R.P. "A simple time-lapse imaging system for use in the classroom: Plants in motion." *Plant Physiol.*, 114(S), 316 (1997).

Jenesen, C.J.P., Ruegger, M., Estelle, M.A., and Hangarter, R.P. "Phototropic and gravitropic responses in auxin-related mutants." 8th International Conference on *Arabidopsis* Research, Madison, WI (1997).

Jenesen, P.J., Hangarter, R.P., and Estelle, M.A. "Affect of light on 1-naphthylphthalamic acid (NPA) induced inhibition of elongation in *Arabidopsis*." *Plant Physiol.*, 114(S), 63 (1997).

Tamot, B. and Hangarter, R.P. "Characterization of a gravity response mutation isolated from the phytochrome B mutant line, hy3-1, in *Arabidopsis thaliana*." European Symposium on Photomorphogenesis, Leicester, England (1997).

Weinig, C. and Hangarter, R.P. "Light-quality cues and leaf architecture in *A. thaliana*." European Symposium on Photomorphogenesis, Leicester, England (1997).

Self-Generating Bending Moments in Root Gravitropism

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NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The main direction of this project remains unchanged. We are still attempting to describe the principal biomechanical parameters of seedling germination. In particular, we are trying to determine the levels of force output of gravitropically responding corn roots. In addition, we are investigating the effects of tip loading on germinating roots. Within the general thrust of this project, however, a new direction has emerged. In our effort to determine the biomechanical parameters surrounding root growth, we have come up with a novel method for the determination of turgor pressures within plant cells. Our method, which is unique in its rapidity and repeatability and may assist workers in a number of fields who may need to determine the mechanical properties of plant cells and tissues. We are pursuing this new interest in parallel with the original goals of the project since it complements them directly and will broaden the base of information which the project yields.

We have developed a novel method for the measurement of turgor pressure in plants. This is the first non-destructive, repeatable, cell-specific method for the determination of this important plant cell growth parameter. The significance of this method lies in the fact that all plant growth and development is driven by the pressure which builds up within the cells. This pressure is known as turgor pressure. Previous methods are either indirect or destructive of the cells being measured. We use an optical method to measure the contact area between a rigid probe and the cell surface. Since the contact area is a direct function of the pressure within the cell, it affords a direct scalar of pressure. This method will be applicable to our overall project by allowing us to relate the gravitropic performance of roots to changes in turgor pressure of surface cells.

This project will continue to yield new understanding of the basic biological process of seed germination and seedling establishment. During the first hours after the emergence of the root from the dormant seed, the root must first determine the direction of the gravity vector; second it must actively bend towards the substrate, (the earth), and third, it must successfully penetrate the earth in order to establish a viable seedling. This research will yield a better understanding of these critical biomechanical processes, enhancing our ability to understand and manipulate the germination process both on Earth and in space, where the principal cue for these processes, namely the gravity vector, may be severely attenuated or lacking. Eventually, this work could be translated into modified agricultural practices and improved germination rates based on a better understanding of the basic biomechanical parameters underlying seedling performance during germination. Immediate benefits include the

development of a new technology for the rapid, non-destructive measurement of cell turgor pressures, an essential measure of water stress, and a critical element in the developmental mechanics of plant growth.

FY97 Publications, Presentations, and Other Accomplishments:

Kuzeja, P. and Lintilhac, P.M. "Elongation rates of *Zea mays* under controlled impedance." (Abstract) AIBS Annual Meeting, Montreal, PQ (1997).

Lintilhac, P.M., Outwater, J.O., and Taylor, H. "A rapid, non-destructive method for the measurement of turgor pressure in exposed plant cells." (Abstract) AIBS Annual Meeting, Montreal, PQ (1997).

Patent In Process, Patent #: Undetermined. Lintilhac, P.M., and Outwater, J.O. "Method and Apparatus for Determining the Turgor Pressure in Plant Cells."

Lynch, T.M. and Lintilhac, P.M. "Mechanical signals in plant development: A new method for single cell studies." (Abstract) AIBS Annual Meeting, Montreal, PQ (1997).

Lynch, T.M. and Lintilhac, P.M. "Mechanical signals in plant development: A new method for single cell studies." *Develop. Biol.*, 181, 246-256 (1997).

Lynch, T.M., Domoszych, D., and Lintilhac, P.M. "A b-one integrin-like protein in higher plant statoliths." *Protoplasma* (In Press).

Lynch, T.M., Lintilhac, P.M., and Domozych, D. "Mechanotransduction molecules in plant gravisensory responses: Amyloplast/statolith membranes contain a b-one Integrin-like protein." (Abstract) AIBS Annual Meeting, Montreal, PQ (1997).

Outwater, J.O., Lintilhac, P.M., and Taylor, H. "A hand-held instrument for field determination of turgor pressure in plants." (Abstract) AIBS Annual Meeting, Montreal, PQ (1997).

Taylor, H.R., Outwater, J.O., and Lintilhac, P.M. "A microscope-based method for the determination of turgor pressures in plant cells." AIBS Annual Meeting, Montreal, PQ (1997).

Gravitropic Signal Transduction in the lazy-2 Tomato Mutant

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Funding:

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Students Funded Under Research: 8

Post-Doctoral Associates: 1

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Task Description:

This research combines approaches from genetics, cell and molecular biology, and plant physiology on a question central to understanding how plants perceive, transduce, and respond to gravity. The *lazy-2* mutant of tomato is unique in that *lazy-2* plants exhibit a completely normal gravitropic response in the dark or under blue light conditions, but the direction of shoot gravicurvature is reversed upon exposure to red light. With the exception of the shoots growing downward, all other phenotypic characteristics of the *lazy-2* mutant are identical to wild-type plants. We have demonstrated that the altered mutant response is regulated by the photoreceptor phytochrome, and our recent evidence indicates that the reversed gravicurvature results from reversal of the lateral redistribution of auxin. We now plan to examine the mechanism of this light-mediated reversal of auxin transport. This will include ultrastructural studies, examination of the expression of auxin-inducible genes, and generation of additional alleles of the *lazy-2* mutation as well as suppressors of that mutation. During the current grant period, we have also begun genetic studies designed to map the *lazy-2* lesion. These efforts will be continued and should result in map-based cloning of the mutated gene. This will provide an important link between red light and control of stem elongation and help to elucidate the mechanism of the plant gravitropic response. The research supports the goals of the Space Biology Program in determining the effects of the interaction of gravity and another environmental factor (red light) on biological systems. A better understanding of the *lazy-2* mutation should lead to well-defined flight experiments which will test the possibility that proper light manipulations can compensate for the absence of gravity in regulating stem development and orientation.

In order to obtain a more precise determination of the path of auxin transport during both the normal seedling gravitropic response and the reversed *lazy-2* curvature, we have developed techniques to examine the expression of auxin-regulated genes. The advantage of this approach is that it should represent not only changes in auxin concentrations, but also any potential changes in auxin sensitivity (e.g., auxin receptors or signal transduction) in response to the gravitropic stimulus. To this end, we have isolated and characterized 11 members of the IAA/Aux family of auxin-regulated, putative transcription factor genes from tomato. The LeIAA (*Lycopersicon esculentum* IAA/Aux) genes fall into the evolutionary and functional classes previously described in *Arabidopsis thaliana*. Using those LeIAA genes which are most responsive to applied auxin and reverse transcription polymerase chain reaction (RT-PCR), we have found that there is indeed increased expression of these auxin-regulated genes on the lower side of upward-bending wild-type seedlings. The same expression pattern was observed for dark-grown *lazy-2* seedlings where the mutant phenotype was not induced. This indicates that auxin concentrations and/or sensitivity do increase on the lower side of horizontally-oriented stems in response to the gravity stimulus, supporting the prevailing Cholodny-Went theory. In *lazy-2* stems where

the reversed gravitropic response (i.e., downward bending) has been induced by red light, we do not see a corresponding reversal in the expression of LeIAA genes (i.e., increased expression on the upper side with respect to gravity). We are currently repeating this result with other auxin-regulated genes; however, if this observation is confirmed, it indicates that in addition to an auxin-dependent gravitropic response mechanism, there is also an auxin-independent one. We are currently preparing to use the technique of *in situ* hybridization to visualize auxin-regulated gene expression at the cellular level in order to both substantiate these findings and to enhance our knowledge of the cellular pathways of auxin transport during gravicurvature.

The literature on plant gravitropism contains conflicting reports as to whether the plant hormone ethylene is also involved in regulating the gravitropic response mechanism. Since red light has been shown to alter ethylene levels, the possibility existed that the red light-induced reversal of gravicurvature in *lazy-2* is mediated by changes in ethylene levels or sensitivity. We have recently found that exogenous ethylene levels do not change in response to either gravistimulation or red light in either *lazy-2* or wild-type plants. The *lazy-2* phenotype can also not be restored to normal by applications of exogenous ethylene. Interestingly, the reduced gravitropic response of an auxin-resistant tomato mutant, *diageotropica*, can be restored to wild-type levels by extremely low levels of exogenously-applied ethylene. This indicates that low levels of ethylene are required for a complete gravitropic response. We have also observed that intermediate levels of ethylene can completely inhibit the normal seedling gravitropic response without significantly inhibiting overall elongation rates of the stem. This indicates a specific rather than non-specific interaction between ethylene and the plant gravitropic response.

The reversal of gravitropic bending in the *lazy-2* mutant is mediated by the red light photoreceptor, phytochrome. Because at least five different phytochrome genes are expressed in tomato, it is of interest to know which of the gene family members are involved in modulating the gravitropic response. In order to address this, we have constructed double and triple mutants in which *lazy-2* plants also lack either phytochrome A (phyA), phyB1, or both. Analysis of the gravitropic response of these plants under different lighting conditions indicate that phyA alone is required for bending under low fluence far-red light, but that in other light environments at least one other phytochrome participates in inducing reversed gravitropism on *lazy-2* plants.

The *lazy-2* gene product obviously plays an important role in integrating the plant growth response to gravity and light. Isolation of the corresponding gene could provide important information as to the gravitropic response mechanism and its regulation by environmental factors. To this end, we have initiated map-based cloning of the *lazy-2* gene. To date, we have determined that the *lazy-2* gene is tightly linked to the centromere of tomato chromosome 5. As there is little recombination in centromeric regions, this may make further map-based cloning by traditional methods difficult. We are currently exploring alternate approaches, including creation of different mapping populations and physical mapping.

In addition to helping define conditions which will be important to proper orientation and development of plants during microgravity conditions, research using the *lazy-2* mutant should contribute to enhanced understanding of basic biological processes on Earth. The fact that the gravitropic response mechanism is not simply broken, but instead reversed, provides an opportunity to test current hypotheses for the tissues and cellular changes underlying curvature in response to gravistimulation. In addition to confirming the basic hypotheses, we have now been able to extend them to include the role of other hormones, light, and an apparently non-auxin regulated mechanism underlying gravicurvature. These findings have important implications for plant growth regulation in both natural and agricultural settings on Earth.

FY97 Publications, Presentations, and Other Accomplishments:

Behringer, F.J. and Lomax, T.L. "Involvement of phytochrome in the reversed gravitropic response of the *lazy-2* tomato mutant." *Plant Physiol.*, 114, 132 (1997).

Behringer, F.J. and Lomax, T.L. "Photobiology and genetics of the *lazy-2* tomato mutant." *ASGSB Bull.*, 10, 44 (1996).

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- Coenen, C. and Lomax, T.L. "Auxin-cytokinin interactions in higher plants: Old problems and new tools." *Trends Plant Sci.*, 2, 351-356 (1997).
- Ellard-Ivey, M., Hopkins, R.B., White, T.J., and Lomax, T.L. "A zucchini calcium-dependent protein kinase gene, cpCPK1; Molecular cloning, characterization, and identification of genes encoding proteins which interact with the cpCPK1 gene product." *Plant Physiol.*, 114, 277 (1997).
- Jones, A.M. and Lomax, T.L. "Photoaffinity labeling with 5-azidoindole-3-acetic acid" in "Methods in Plant Biochemistry and Molecular Biology." Edited by: Dashek, W.K. CRC Press, Boca Raton, FL, pp 115-132 (1997).
- Kim, K-H. Light and gravity interaction in the *lazy-2* tomato mutant. (Thesis) Oregon State University (1997).
- Lomax, T.L. "Molecular genetic analysis of plant gravitropism." *ASGSB Bull.*, 10, 75-82 (1997).
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- Madlung, A., Behringer, F.J., Kim, K-H., and Lomax, T.L. "The kinetics of stem bending and auxin redistribution in the tomato *lazy-2* mutant." *ASGSB Bull.*, 10, 72 (1996).
- Madlung, A., Behringer, F., Nebenführ, A., and Lomax, T.L. "Auxin-induced gene expression during the gravitropic response of tomato hypocotyls." *ASGSB Bull.*, 11, 7 (1997).
- Miller, D., Hable, W., Gottwald, J., Ellard-Ivey, M., Demura, T., Lomax, T., and Carpita, N. "Connections: The hard wiring of the plant cell for perception, signaling, and response." *Plant Cell*, 9, 2105-2117 (1997).
- Nebenführ, A. Characterization of the LeIAA family of auxin-regulated genes in tomato. (Dissertation) Oregon State University (1997).
- Nebenführ, A. and Lomax, T.L. "Tissue-specific expression of LeIAA genes in tomato." *Plant Physiol.*, 114, 62 (1997).

Molecular Cloning of the Arabidopsis thaliana AGR1 Locus

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Post-Doctoral Associates: 2

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Task Description:

Plants orient the growth of their primary organs according to the gravity vector. In roots, a gravity stimulus induces a reorientation of growth in the elongation zone, provided the root cap is present. The long-term objectives of our research are to define the molecular events involved in gravity sensing and signal transduction by the root of *Arabidopsis thaliana*.

We have identified several *A. thaliana* mutants affected in root gravitropism. Two of them are likely to be affected in gravity sensing and/or early phases of gravity signal transduction. *agr1* mutants are characterized by a specific defect in root gravitropism and ethylene resistance, while *arg1* mutants present a defect in both root and hypocotyl gravitropism. Unlike most other agravitropic mutants, *agr1* and *arg1* mutants show no pleiotropic phenotypes. *agr1* was mapped on the South arm of chromosome V, while *arg1* was localized to the South arm of chromosome I. The present proposal is aimed at cloning and characterizing molecularly the *agr1* and *arg1* loci, using a combination of chromosome walking and transposon tagging strategies.

To better characterize the molecular functions of these loci, we will also clone and sequence the corresponding cDNAs and mutant alleles of both loci, and we will characterize the corresponding proteins. Also, we will characterize the pattern of expression of both genes, and we will localize the corresponding proteins within the plant cells and organs. The genetic, physiological, and molecular characterization of these mutations should provide important information expanding our understanding of the molecular mechanisms underlying gravity sensing and transduction in plant roots.

Mutations at the *AGR1* locus of *Arabidopsis thaliana* result in altered root gravitropism (Bell and Maher, 1990). Hypocotyl gravitropism is also altered in *agr1* mutant seedlings early after germination, but becomes wild-type after only a few days of growth. Last year, we reported that the strongest *agr1* alleles confer increased root resistance to exogenous ethylene. We have now shown that the same strong *agr1* alleles are responsible for increased sensitivity to exogenous auxin, suggesting a possible role for *AGR1* in polar auxin transport. We have pursued experiments aimed at cloning the *AGR1* locus, using a combination of chromosome walking and DNA transformation strategies. This allowed us to identify a 30-kb fragment of genomic DNA encompassing a region of chromosome 5 that overlaps with *AGR1*. That cloned fragment complemented the agravitropic phenotype when transformed into homozygous *agr1* plants, demonstrating that it carries the wild type *AGR1* locus (Sedbrook et al., 1997). Part of that fragment was shown to be deleted in one *agr1* allele, and a 12-kb

subfragment thereof was shown to detect, by Northern blot analysis, two transcripts in wild type seedlings. One of these transcripts was shown to be either absent, less abundant, or altered in plants homozygous for several strong alleles of *agr1*, strongly suggesting that it derives from the *AGR1* locus. We are in the process of sequencing that fragment of genomic DNA. Similarly, we have identified a cDNA hybridizing to that subfragment, and are in the process of purifying it for further analysis (Chen, Hilson, Rosen, Sedbrook, and Masson, unpublished data).

Having cloned the *AGR1* gene, we plan on defining its wild-type sequence and characterizing the sequence of several mutant alleles (we have now identified 15 *agr1* alleles, constituting a nice allelic series with regard to allelic strength) in order to initiate a structure-function analysis of the locus. Similarly, we are setting up to define the pattern of *AGR1* expression in wild type tissues under gravity-induced and uninduced conditions, to direct antibodies against the *AGR1* protein, and to initiate a new screen for second-site mutations that modify (enhance or suppress) the *agr1* phenotype (Chen and Masson, unpublished data).

agr1 mutations confer altered root and hypocotyl gravitropism, with no pleiotropic phenotypes (Masson et al., 1993). We have also progressed in the molecular characterization of the *Arabidopsis thaliana* *AGR1* locus. We have cloned that locus by chromosome walking, and shown it to potentially encode a 43 kd protein with multiple functional domains. The amino terminal section of the *ARG1* protein is similar to the J domain found in all dna-J-like proteins, while its amino-terminal half carries a domain similar to coiled-coil regions found in several microfilament- or microtubule-binding proteins. A domain located between the J domain and the coiled-coil region possesses all the characteristics of a transmembrane domain. These data suggest that the *ARG1* protein interacts with the plant cytoskeleton, as well as possibly with other proteins through its J domain (Sedbrook et al., 1997).

Interestingly, *ARG1* is highly expressed in all tissues of the plant. That high level of ubiquitous expression appears to contradict our conclusion (derived from phenotypic analysis of mutant seedlings) that *ARG1* is specifically involved in root and hypocotyl gravitropism, and suggests that other possible *ARG1* functions are masked by gene redundancy in *Arabidopsis thaliana*. Experiments are in progress to determine the cellular and subcellular localization of the *ARG1* protein in wild type and mutant plants under induced and uninduced conditions and to identify genetically and biochemically other gene products that interact with it (Sedbrook and Masson, unpublished data).

In the long term, we hope that this combination of genetic, molecular, and physiological analysis of the *agr1* and *agr1* mutations will provide important clues and tools for the characterization of the mechanisms involved in gravity sensing, signal transduction, and response in plant roots.

Plant organs use the gravity vector as a cue to define the vector of their growth. That response, gravitropism, allows for shoots to grow upward and for roots to grow downward. This is of major interest for agricultural productions as it allows crop plants to orient their organs optimally for photosynthesis and for water and nutrient uptake. It also allows crop shoots to resume vertical growth after being prostrated by the action of wind and rain, thereby maintaining seeds away from soil moisture and available for mechanical harvest.

FY97 Publications, Presentations, and Other Accomplishments:

Masson, P.H. "Root Gravitropism and Waving in *Arabidopsis thaliana*." Seminar, University of Wisconsin-Madison, Laboratory of Genetics (October 31, 1996).

Masson, P.H. "Root Gravitropism and Waving in *Arabidopsis thaliana*." Seminar, Marquette University-Milwaukee (November 22, 1996).

Pearlman, R., Silo-Suh, L., Hilson, P., and Masson, P.H. "Characterization of an *Arabidopsis thaliana* mutant developing dampened root waves on tilted agar plates." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Rutherford, R., Gallois, P., McEvoy, J., and Masson, P.H. "A startling connection between the indolic biosynthesis pathway and root waving: The *wvc1* mutation of *ASA1*." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Sedbrook, J. and Masson, P. "Molecular Characterization of the *ARG1* Gene of *Arabidopsis thaliana*." (Poster) Annual Meeting of the David and Lucile Packard Foundation / Fellowships in Science and Engineering, Monterey, CA (September 3 - 6, 1997).

Sedbrook, J. and Masson, P.H. "The *ARG1* (Altered Response to Gravity) Gene of *Arabidopsis thaliana* Encodes a Novel DnaJ-Like Protein With Predicted Actin Binding." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Sedbrook, J., Hilson, P., Chen, R., Caspar, T., and Masson, P. "Molecular cloning and characterization of the *AGR1* and *ARG1* loci involved in gravitropism in *Arabidopsis thaliana*." Annual Meeting of the American and Canadian Societies of Plant Physiologists, Vancouver, Canada (August 2 - 6, 1997).

Molecular Genetics of Root Thigmoresponsiveness in Arabidopsis thaliana

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No Co-Is Assigned to this Task

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Task Description:

The direction of root growth is dictated by a variety of environmental factors. These include the vectors of gravity (gravitropism) and light (phototropism), gradients in water (hydrotropism), temperature (thermotropism), ions, chemicals (chemotropism), and oxygen (oxotropism). At any time, a root defines the pattern of its growth by integrating the information derived from these environmental stimuli. The efficiency of this process conditions the levels and quality of plant productions.

While growing towards better microenvironments, roots also encounter physical obstacles. Hence, they have to detect such obstacles and respond to their presence by reorienting their growth if they want to avoid them. The general objectives of this proposal are to understand the molecular mechanisms associated with touch sensing and response in plant roots. We will use molecular genetic approaches in *Arabidopsis thaliana* to identify, clone, and characterize genes involved in touch sensing and response by plant roots. Various collections of T-DNA (Ds) insertional mutants of *Arabidopsis thaliana* will be screened for mutants affected in their ability to change the direction of growth of their roots upon touch stimulation, as described (Okada and Shimura, 1990, *Science* 250: 274-276). The corresponding genes will be cloned and characterized. Their pattern of expression will be determined, and the predicted sequence of the corresponding protein will be analyzed and searched for homologies with other known proteins in data bases. Each mutant will be subjected to a combination of genetic, molecular, physiological, and cytological assays aimed at better characterizing the function(s) of the tagged gene. In the long term, the data obtained for each mutant will allow the progressive development of a pathway for transduction of the touch signal towards growth response in roots.

When *Arabidopsis thaliana* seedlings are grown on agar surfaces tilted backward, their roots develop a wavy pattern of growth in response to a combination of gravity, touch, and other surface-derived stimuli (Okada and Shimura, 1990; Rutherford and Masson, 1996). That growth process is accompanied by a reversible rotation of the root tip about its axis. It involves a succession of left-handed and right-handed circumnutation-like processes (Rutherford and Masson, 1996).

To identify genes involved in the response of *Arabidopsis* roots to a combination of gravity and touch stimulation, we have identified a number of mutants developing altered root waves on tilted agar surfaces. Some of them develop no waves on such surfaces, others develop compressed waves, and yet others develop concentric circles rather than waves under those conditions. The purpose of this project is to characterize mutations which affect root waving without altering root gravitropism.

This year, we have pursued our analysis of four wavy root mutants: Two developing no waves on tilted agar surfaces (*wvd2* and *wvd6*), and two developing compressed root waves under these conditions (*wvc1* and *wvc16*).

The roots of homozygous *wvd2* mutant seedlings develop no waves on tilted agar surfaces. They also develop a thickened root phenotype due to an increase in cell diameter rather than a change in cell number. That secondary phenotype is, however, not completely penetrant. *wvd2* mutant seedlings were identified in a collection of *Arabidopsis* seedlings carrying transposed copies of a *Ds* transposable element (Pearlman et al., 1997). Co-segregation analysis performed on more than 150 segregating F2 progeny indicates that *wvd2* co-segregates with a *Ds* element. Additionally, revertants were obtained from a population of plants carrying *wvd2* at the homozygous state along with a source of transposase, and these revertants were shown to be heterozygous for *wvd2* as well as for the *Ds* insertion. Interestingly, the wild type progeny from these revertants carry at least one copy of a modified *WVD2* allele containing a typical footprint of *Ds* excision. Taken together, these data strongly suggest that *wvd2* is tagged by a *Ds* element.

We have cloned and sequenced genomic sequences flanking the original *Ds* element in *wvd2* plants. We have also cloned and sequenced a cDNA hybridizing to that clone (Pearlman et al., 1997). The *WVD2* gene encodes a short glutamate-rich protein with no obvious known functional domains in it. The *WVD2* protein is orthologous to several other proteins found in the EST databases, including three from *Arabidopsis thaliana* and one from rice. All five sequences are very similar to each other, maintaining at least 80% identity within and across species (Pearlman and Masson, unpublished data). This suggests that the *WVD2* gene is a member of a multigene family in *Arabidopsis thaliana*, and the sequence conservation found amongst species suggests that it plays an important functional role in plants. Experiments are currently underway to define the pattern of *WVD2* gene expression, to direct antibodies against the protein in order to immunolocalize it in the cells, and to identify mutations in some of the other *Arabidopsis thaliana* genes belonging to the same gene family (Pearlman, Silo-Suh, and Masson, unpublished data).

The *wvd6* mutation also confers a dampened root wave phenotype on tilted agar surfaces. Additionally, *wvd6* promotes a decrease in the rate of mutant root growth. Co-segregation analysis of over 150 progeny has shown that the mutation is probably due to the insertion of a promoter-trap T-DNA in the *HSF4* locus of *Arabidopsis thaliana*. *HSF4* codes for heat-shock-type transcription factor. Such factors were shown to activate gene expression in response to heat shock, as well as in response to other stimuli in animal cells (transition heavy metals, pathophysiological conditions, or developmental processes: Morimoto et al., 1992). Interestingly, *wvd6* mutant plants show no evidence of thermotolerance defect (Sedbrook and Masson, unpublished data). The promoterless *GUS* reporter gene carried by the mutagenic T-DNA is expressed specifically, although poorly, in the root cap, and not in other plant tissues. Experiments are in progress to determine if *wvd6* is indeed a mutation of *HSF4* (as opposed to a mutation tightly linked to *HSF4*), to determine if the pattern of *HSF4* expression is truly reported by the promoterless *GUS* reporter gene, and to localize the protein in transformed and untransformed cells (Sedbrook and Masson, unpublished data). Similarly, we have generated a collection of cDNAs differentially expressed in wild type and *wvd6* mutant root tips in response to wave induction, with the hope of identifying some genes whose expression is directly or indirectly controlled by *HSF4* (Schulz, Sedbrook, and Masson, unpublished data).

Although mutations resulting in dampened root waves on tilted agar surfaces are likely to define genes whose functions are essential for root waving, mutations resulting in compressed root waves are likely to provide clues on the mechanisms involved in regulating the circumnutation-like processes which define that phenotype. This year, we have completed our analysis of *wvc1*, a mutation which affects the *ASA1* gene coding for one subunit of anthranilate synthase, an enzyme of the TRP biosynthetic pathway (Rutherford et al., submitted for

publication). Chemical rescue experiments have been conducted, suggesting that a TRP-derived molecule other than IAA is probably an important regulator of the circumnutation-like processes that condition the shape of the waves on tilted surfaces. Biochemical quantifications of free amino acid levels in plants have shown that tissue extracts from wild type and mutant seedlings or roots contain similar levels of free TRP. These results suggest that the levels of free TRP drop in the mutant when compared to the wild-type in only a small group of cells at the tip of the root, or that a change in the flux of metabolites through the pathway results in a decrease in the production of a TRP-derived regulatory molecule involved in the regulation of root waving (Rutherford et al., 1997).

We have also initiated the molecular characterization of *wvc16*, a mutation which confers a compressed root wave phenotype on tilted surfaces, along with the formation of kinks in inflorescence stems, longitudinal and lateral curling of the leaves, and curling in the siliques. All shoot phenotypes are exaggerated if one inflorescence stem is severed from the plant, suggesting that the phenotype may reflect an inability for the plant to cope with fast changes in water potential. *wvc16* mutant seedlings were identified in a collection of *Arabidopsis* plants carrying transposed copies of *Ds*. Preliminary data indicate that the mutation co-segregates with a *Ds* element in about 125 segregating F2 progeny. Therefore, we are attempting to identify revertants of the mutation in the progeny of homozygous mutant seedlings, to demonstrate that the mutation is indeed caused by the insertion of a *Ds* element in the genome. We have also cloned genomic sequences flanking that *Ds* insert, and are currently in the process of characterizing them (Schulz, Hilson, and Masson, unpublished data).

A genetic, physiological, and morphological characterization of these mutants, along with that of several other dampened and compressed root wave mutants also identified during our screens, should provide important clues for our understanding of the molecular processes underlying root waving in response to gravity, touch, and other surface-derived stimuli in plants.

In soil, roots grow toward environments which constitute a good source of mineral ions and water as well as a good anchorage for the plant. They do so by altering the pattern of their growth in response to numerous environmental cues (gravity, light, gradients in water, ions, chemicals, temperature, and oxygen). However, while growing toward optimal soil environments, roots necessarily encounter obstacles in their path (soil particles, rocks, etc.). Consequently, they have acquired ways to modify the vector of their growth in response to such obstacles (thigmotropism). Clearly, this system conditions the level of plant productions by allowing each plant to better utilize its environment.

Because the vector of root growth is determined by an integrated response to several environmental cues, one has to understand the involvement of each one of these cues in the final, integrated growth response of the plant. One also has to understand how the responses to several environmental cues applied at the same time are integrated into a single pattern of growth if one wants to design strategies allowing to better control the process. This is especially true if one wants to better control the pattern of root growth in microgravity environments where one essential player, gravity, is missing. Hence, the long-term objective of our research is aimed at understanding the mechanisms by which roots sense and respond to mechanical stimulation, and how this response is affected by other environmental cues, such as gravity.

FY97 Publications, Presentations, and Other Accomplishments:

Batiza, A.F., Schultz, T., and Masson, P.H. "Yeast respond to hypotonic shock with a calcium pulse." *J. Biol. Chem.*, 271, 23357-23362 (1996).

Masson, P.H. "Root Gravitropism and Waving in *Arabidopsis thaliana*." Seminar, University of Wisconsin-Madison, Laboratory of Genetics (October 31, 1996).

Masson, P.H. "Root Gravitropism and Waving in *Arabidopsis thaliana*." Seminar, Marquette University-Milwaukee (November 22, 1996).

Pearlman, R., Silo-Suh, L., Hilson, P., and Masson, P.H. "Characterization of an *Arabidopsis thaliana* mutant developing dampened root waves on tilted agar plates." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Rutherford, R., Gallois, P., McEvoy, J., and Masson, P.H. "A startling connection between the indolic biosynthesis pathway and root waving: The *wvc1* mutation of *ASA1*." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Sedbrook, J. and Masson, P. "Molecular Characterization of the *ARG1* Gene of *Arabidopsis thaliana*." (Poster) Annual Meeting of the David and Lucile Packard Foundation / Fellowships in Science and Engineering, Monterey, CA (September 3 - 6, 1997).

Sedbrook, J. and Masson, P.H. "The *ARG1* (Altered Response to Gravity) Gene of *Arabidopsis thaliana* Encodes a Novel DnaJ-Like Protein With Predicted Actin Binding." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).

Sedbrook, J., Hilson, P., Chen, R., Caspar, T., and Masson, P. "Molecular cloning and characterization of the *AGR1* and *ARG1* loci involved in gravitropism in *Arabidopsis thaliana*." Annual Meeting of the American and Canadian Societies of Plant Physiologists, Vancouver, Canada (August 2 - 6, 1997).

The Role of Actin Cytoskeleton in Auxin Transport and Gravitropism

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Students Funded Under Research: 6

Post-Doctoral Associates: 0

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Task Description:

Although it has been more than sixty years since the first experiments suggested that changes in auxin transport may be important in plant gravitropism, the mechanisms by which auxin transport is regulated during gravitropism remain unclear. The polarity and quantity of auxin transport are believed to be controlled at the site of auxin efflux. The experiments in this grant will examine the biochemistry of the auxin efflux carrier and the relationship between auxin transport and root growth and gravitropism.

A critical step in the dissection of the regulatory pathway of auxin transport is the characterization of the auxin transport inhibitor binding protein and determination of the mechanisms by which this regulatory protein controls auxin efflux from plant cells. The experiments in this proposal will examine this protein and its interaction with the actin cytoskeleton to strengthen preliminary evidence suggesting that the efflux carrier interacts with the cytoskeleton and to explore the use of this interaction as a method for isolation of the efflux carrier. Experiments will also examine the dependence of auxin transport and gravity response on an intact actin cytoskeleton.

The second approach of this work will examine auxin transport in roots of wild-type and mutant *Arabidopsis* plants in order to provide more information on the relationship between auxin transport and root growth and gravity response. These experiments will ask about the polarity of auxin transport that controls gravity response, elongation, and lateral root development in wild-type plants. This information will be used to characterize mutant plants that have alterations in auxin transport and/or gravity response in order to understand the relationships between these processes.

The first goal of this proposed work was to examine the relationship between auxin transport and the actin cytoskeleton. A second publication providing biochemical evidence indicating that the auxin transport inhibitor binding protein associates with the actin cytoskeleton is now in press. These experiments showed that treatments which specifically alter the polymerization state of the actin cytoskeleton suggest that this protein partitions with actin in all procedures, but can be easily separated from the microtubule network. Additional experiments have examined whether an intact actin cytoskeleton is required for auxin transport. In both zucchini hypocotyls and tobacco tissue culture cells, treatment with cytochalasin D alters auxin efflux, suggesting that

the integrity of the cytoskeleton does regulate auxin efflux. Additional experiments are in progress to test the effects of other drugs that alter actin or microtubule polymerization. Finally, procedures have been developed to purify actin from zucchini hypocotyls and experiments have been performed to demonstrate that this actin is in a native conformation that will allow repolymerization of actin filaments. This actin has been used to prepare both G- and F-actin affinity columns. The F-actin columns have been shown to retain auxin transport inhibitor protein binding activity, as compared to the G-actin columns. These procedures are being optimized to maximize recovery of NPA binding activity.

The second goal of the project is to provide information on auxin transport in the roots of *Arabidopsis thaliana* in order to understand how transport may be altered during root gravitropism. Experiments have been performed to unlink the two polarities of auxin transport and have found that only auxin transported from the shoot into the root is required for lateral root development, whereas auxin transported from the tip to the base of the root plays no role in this process. In contrast, tip to base transport of auxin is required for gravity response, growth, and root waving. Experiments are currently in progress to determine if auxin transport from the shoot into the root is the source of auxin that is transported from the tip to the base. Additional experiments are being performed in *Arabidopsis* plants with specific genetic lesions. One set of experiments has been initiated to test the hypothesis that flavonoids are natural inhibitors of auxin transport and to determine if flavonoid abundance or distribution affects gravity response. These experiments have indicated that in a plant which makes no flavonoids, due to a null mutation in the gene encoding the first enzyme in flavonoid biosynthesis, there is an increased rate of auxin transport and an altered phenotype consistent with elevated auxin transport. This plant is still able to respond to gravity, and experiments are in progress to determine if the rate of transport is altered in the mutant. A collaboration has been initiated to characterize a series of mutants isolated in the laboratory of Dr. Mark Estelle. These mutants are altered in sensitivity to auxin transport inhibitors and some of these mutants have altered gravity response. We have been examining the levels of auxin transport inhibitor binding protein in these mutants and found the *tir3* mutation reduces auxin transport inhibitor binding activity in addition to have reduced auxin transport. Analyses of other mutants are also in progress, in order to provide greater understanding of the relationship between auxin transport and gravity response.

The goal of the research supported by this grant is to increase understanding of a basic biological process, the response of plants to gravity. This study focuses on the role of transport of a class of plant hormones, the auxins, in gravity response. The long-term goals of this study include understanding how one of the proteins which controls auxin transport functions and how gravity stimuli may affect this protein's function to allow changes in auxin transport and changes in growth in response to gravity. A clearer understanding of the mechanisms by which plants respond to changes in the vector of gravity may provide important insight into predicting how plants will grow in the absence of gravity and may facilitate the design of experiments to study plant response during space flight.

FY97 Publications, Presentations, and Other Accomplishments:

Brady, S.R., Dixon, M.W., Cyr, R.J., Fisher, D.D., and Muday, G.K. "BY2 protoplasts as a model for NPA sensitive auxin transport and gravitropism." (Abstract) ASGSB Bull., 10, 78 (1996).

Brady, S.R., Reed, R.C., and Muday, G.K. "The role of basipetal auxin transport in root elongation, gravitropism, and waving in *Arabidopsis thaliana*." ASGSB Bull. (In Press).

Brown, D. and Muday, G.K. "Testing the hypothesis that flavonoids regulate auxin transport *in vivo*." 8th International Conference on *Arabidopsis* Research (1997).

Brown, D.E., Reed, R.C., and Muday, G.K. "Polar auxin transport and gravitropism in *Arabidopsis thaliana*." (Abstract) ASGSB Bull., 10, 38 (1996).

Butler, J.A., Hu, S., Brady, S.R., Dixon, M.W., and Muday, G.K. "*In vitro* and *in vivo* evidence for actin association of the NPA binding protein." Plant J. (In Press).

Hu, S., Dixon, M.L., and Muday, G.K. "Development of a procedure to depolymerize the actin cytoskeleton for purification of associated proteins." (Abstract) ASGSB Bull., 10, 27 (1996).

Luciano, R.L. and Muday, G.K. "Characterization of gravity dependent growth in *Fucus zygotes*." (Abstract) ASGSB Bull., 10, 26 (1996).

Muday, G., Brown, D., Cowan, L.L, Robertson, D., and Wyatt, S.E. "Gravity perception and response in the the inflorescence stems of *Arabidopsis thaliana*." ASGSB Bull. (In Press).

Muday, G.K. "The role of auxin transport and the actin cytoskeleton in plant gravity response." NSCORT Meeting, Kennedy Space Center, FL (February, 1997).

Muday, G.K. and Reed, R.C. "Inhibition of auxin movement from the shoot into the root inhibits lateral root development in wild-type *Arabidopsis thaliana* and alf1-1." 8th International Conference on *Arabidopsis* Research (1997).

Reed, R.C. and Muday, G.K. "*Arabidopsis* lateral root development requires auxin from the shoot." (Abstract) Plant Phys., 111S, 590 (1996).

Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G., and Estelle, M. "Reduced NPA-binding in the *tir3* mutant of *Arabidopsis* is associated with a reduction in polar auxin transport and diverse morphological defects." Plant Cell, 9, 745-757 (1997).

Wyatt, S.E., Brown, D., Robertson, D., and Muday, G.K. "The gravitropic response of the inflorescence stems of *Arabidopsis*." (Abstract) Plant Physiol., 114S, 614 (1997).

Microgravity Effects on Early Reproductive Development in Plants

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Students Funded Under Research: 2

Post-Doctoral Associates: 2

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Task Description:

The ability of plants to reproduce sexually in microgravity has been in question since early investigations by the Soviets. Using a range of plant species and growing conditions, they reported a general failure in plant development during the reproductive stage. In our first flight experiment which probed early events in the reproductive development in *Arabidopsis thaliana*, we found both pollen and ovule development were disrupted by space flight conditions. The object of the current proposal is to continue the investigation of our flight material and to further elucidate mechanisms leading to these reproductive lesions during space flight through additional ground-based and flight experiments. Because the foliage of the flight material had significantly lower carbohydrate content than the ground control, we will investigate the possibility that reproductive development failed due to lack of sufficient carbohydrate supply in flight. By investigating possible indirect effects of microgravity on environmental factors such as gas and solute movement, we should be able to determine whether these reproductive problems are a direct result of the microgravity environment, an indirect result, or a result of some other aspect of the space flight environment. The findings will be significant not only in terms of advancing our basic knowledge of space biology, but also to provide information for those scientists who intend eventually to assist human habitation of space with a plant-based food supply.

The role of subambient oxygen concentrations in development of seeds was studied extensively in the model plant *Arabidopsis thaliana*. Using pre-mixed gases and individual flow-through chambers, it was possible to compare multiple oxygen concentrations side by side through full-life exposure of numerous plants to the test gas concentrations. Material has been analyzed at the microscopic, ultrastructural, and biochemical levels to understand the complex factors that combine in oxygen control of seed development. With additional funding from the Louisiana Space Consortium, it was possible to utilize unique facilities at the Biocurrents Research Center at the Marine Biological Laboratory in Woods Hole, MA, to measure oxygen concentrations around developing seeds inside siliques by using glass oxygen microelectrodes that could be inserted through the silique wall. These unique measurements let us understand the true oxygen environment that the developing seeds are exposed to and how light changes the oxygen concentration around the seeds through silique wall photosynthesis. The results of these experiments were presented at several national meetings and as publications.

In general, this work will increase our understanding of plant growth and development as it is affected by atmospheric composition. The low O₂ studies will provide baseline information that will allow future researchers to grow plants in space at oxygen levels lower than current Earth levels, thereby decreasing O₂ requirements for a future space plant growth facility. The results of the altered CO₂ studies will help future researchers determine if plants can grow and reproduce in the elevated levels of CO₂ typical of a spacecraft environment. Because plants consume CO₂ (high concentrations of CO₂ are lethal for humans), plants could be used as a "CO₂ scrubber" in space environments. In terms of Earth-based benefits, the CO₂ studies will contribute information to the growing knowledge base related to the effects of the rising level of CO₂ in the Earth's atmosphere caused by anthropogenic and natural activities. The O₂ and CO₂ studies may also benefit controlled atmosphere-based industries in horticulture and ornamental floriculture.

FY97 Publications, Presentations, and Other Accomplishments:

- Crispi, M.L., Porterfield, D.M., Murgia, M., and Musgrave, M.E. "Role of metabolic gases in reproductive failure under spaceflight conditions: Ground based studies with *Arabidopsis*." Life Supp. Biosphere Sci. (In Press).
- Kuang, A., Crispi, M.L., and Musgrave, M.E. "Control of seed development in *Arabidopsis thaliana* by atmospheric oxygen." Plant, Cell & Environ. (In Press).
- Kuang, A., Xiao, Y., and Musgrave, M.E. "Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions." Ann. Bot., 78, 343-351 (1996).
- Musgrave, M.E., Kuang, A., and Matthews, S.W. "Plant reproduction during spaceflight." Planta, 203, S177-S184 (1997).
- Musgrave, M.E., Kuang, A., and Porterfield, D.M. "Plant reproduction in spaceflight environments." ASGSB Bull., 10, 83-90 (1997).
- Porterfield, D.M. Physiological responses to nutrient delivery systems in microgravity. (Dissertation) Louisiana State University (1996).
- Porterfield, D.M. and Musgrave, M.E. "Oxytropism in plant roots." (Abstract) ASGSB Bull., 10, 23 (1996).
- Porterfield, D.M., Crispi, M.L., and Musgrave, M.E. "Changes in soluble sugar, starch, and alcohol dehydrogenase in *Arabidopsis thaliana* exposed to N₂ diluted atmospheres." Plant Cell Physiol. (In Press).
- Porterfield, D.M., Matthews, S.W., Daugherty, C.J., and Musgrave, M.E. "Spaceflight exposure effects on transcription, activity and localization of alcohol dehydrogenase in the roots of *Arabidopsis thaliana*." Plant Physiol., 113, 685-693 (1997).
- Ramonell, K.M., Crispi, M.L., and Musgrave, M.E. "Changes in stomatal density in *Arabidopsis thaliana* (L.) Heynh. grown under low oxygen atmospheres." (Abstract) ASGSB Bull., 10, 17 (1996).

Calcium/Calmodulin-mediated Gravitropic Response in Plants

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Funding:

UPN/Project Identification: 199-40-57-50

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Students Funded Under Research: 5

Post-Doctoral Associates: 2

FY 1997 Funding: \$ 121,700

Task Description:

Calmodulin, a Ca^{2+} -binding multifunctional regulatory protein, is the primary transducer of the intracellular Ca^{2+} signal. The goal of this investigation is to study the role of calmodulin isoforms and calmodulin-binding proteins in plant development with emphasis on gravity signal transduction. We have cloned and characterized several calmodulin isoforms and studied their responsiveness to environmental signals such as gravity, light, touch, temperature, stress, and changes in free Ca^{2+} concentration (*Proc. Natl. Acad. Sci.* 86: 3644-3648, 1989; *Plant Mol. Biol.* 27: 693-703, 1995). Transgenic plants carrying one of the isoforms (PCM-1) showed dramatic differences in their responsiveness to environmental conditions which was characterized by altered growth and developmental patterns. To study the role of PCM-1 in signal transduction, transgenic plants were produced carrying the PCM-1 promoter fused to the β -glucuronidase (GUS) reporter gene. GUS expression was developmentally regulated and signal responsive, indicating a positive correlation between the expression of PCM-1 and GUS mRNAs. To further understand the role of calmodulin and its target proteins in gravity signal transduction, two novel genes that encode for calmodulin-binding proteins (TCK1 and CCaMK) were cloned. TCK1 is a kinesin-like gene that encodes for a protein containing a motor domain that is conserved among kinesin proteins. Kinesin is a mechanochemical microtubule-based motor protein that is known to convert the chemical energy stored in ATP into mechanical force. The presence of a novel calmodulin-binding domain in the motor domain of TCK1 makes it distinctly different from all other plant and animal kinesin-like proteins (*Plant Mol. Biol.*, 31: 87-100, 1996). The gravitropic response in transgenic plants carrying the antisense construct of TCK1 was slower than the controls, suggesting a role for TCK1 in gravity signal transduction. Ca^{2+} /CaM-regulated protein phosphorylation is involved in amplifying and diversifying the action of Ca^{2+} -mediated signals. CCaMK, a chimeric Ca^{2+} /calmodulin-dependent protein kinase with a neural visinin-like Ca^{2+} -binding domain, was cloned and characterized (*Proc. Natl. Acad. Sci.* 92: 4897-4901, 1995; *J. Biol. Chem.* 271: 8126-8132, 1996). CCaMK is characterized by the presence of a catalytic domain, a CaM-binding domain, and a visinin-like Ca^{2+} -binding domain in a single polypeptide making it distinctly different from other known plant and animal kinases. We intend to study the temporal and spatial regulation of calmodulin and its binding proteins. Transgenic plants will be used to study the effects of altered levels of calmodulin, TCK1, and CCaMK on plant responses to gravity, light, and touch. The knowledge gained from this investigation will be useful in controlling plant growth under microgravity conditions.

Transgenic plants carrying sense and antisense constructs of the calmodulin isoform (PCM-1) have shown significant alterations in growth characteristics. Furthermore, these transgenic plants also show differences in

their responsiveness to changing environmental conditions as compared to control plants. Transgenic plants carrying the TCK1 and the CCaMK genes are also being studied to gain a better understanding of calcium-mediated signaling in plants.

We have carried out *in situ* hybridization and immunolocalization experiments to study the expression of PCM-1, CCaMK, and TCK1. The mechanisms of activation of CCaMK by calcium and calcium/calmodulin were investigated using various deletion mutants. The use of deletion mutants of CCaMK lacking either one, two, or all three calcium-binding EP hands indicated that all three calcium-binding sites in the visinin-like domain were crucial for maximum kinase activity. As each calcium-binding EP hand was deleted, there was a gradual reduction in kinase activity from 100% to 4%. Another mutant (amino acids 1-322) which lacks both the visinin-like domain containing three EF hands and the calmodulin-binding domain was constitutively active, indicating the presence of an autoinhibitory domain around the calmodulin-binding domain. By using various synthetic peptides and the constitutively active mutant, we have shown that CCaMK contains an autoinhibitory domain within the residues 322-340 that overlaps its calmodulin-binding domain. Kinetic studies with both ATP and the GS peptide substrate suggest that the autoinhibitory domain of CCaMK interacts only with the peptide substrate binding motif of the catalytic domain, but not with the ATP-binding motif. The structural features of CCaMK and mammalian CAMKI were studied by homology modeling. These studies revealed that the kinase and calmodulin-binding domain of these two kinases are similar. However, the visinin-like domain of CCaMK may function as a calcium sensor element in mediating kinase activity.

Studies on the role of calcium and calmodulin in gravity signal perception and transduction will help us to gain a better understanding of how plants respond to changing environmental conditions. Calcium and calmodulin have been shown to regulate diverse physiological processes in plants. We have produced transgenic plants with altered growth characteristics by manipulating the expression of genes that encode for one of the calmodulin isoforms (PCM-1) and the calmodulin-binding proteins (TCK1 and CCaMK). We have already carried out field trials of one set of transgenic plants carrying the PCM-1 gene. The commercial implications of the altered growth characteristics of these plants are being investigated. A patent was obtained in 1996 for this set of transgenic plants.

FY97 Publications, Presentations, and Other Accomplishments:

Liu, Z., Xia, M., and Poovaiah, B.W. "Isoforms and genomic structure of chimeric calcium/calmodulin-dependent protein kinase from tobacco." *Plant Physiol., Suppl.* Vol. 114(3), 276 (1997).

Poovaiah, B.W., Takezawa, D., and Han, T-J. "Regulated expression of a calmodulin isoform alters growth and development in potato." *J. Plant Physiol.*, 149, 553-558 (1996).

Ramachandiran, S., Takezawa, D., Wang, W., and Poovaiah, B.W. "Functional domains of plant chimeric calcium/calmodulin-dependent protein kinase: Regulation by autoinhibitory and visinin-like domains." *J. Biochem.*, 121, 984-990 (1997).

Ramachandiran, S., Wang, W., and Poovaiah, B.W. "Functional domains of plant chimeric calcium/calmodulin-dependent protein kinase: Regulation by autoinhibitory and visinin-like domains." *Plant Physiol., Suppl.* Vol 114(3), 276 (1997).

Takezawa, D. and Liu, Z.H. "Cloning and characterization of genes encoding calmodulin-binding proteins." *ASGSB Bull.*, 10(1), 54 (1996).

Takezawa, D., Patil, S., Bhatia, A., and Poovaiah, B.W. "Calcium-dependent protein kinase genes in corn roots." *J. Plant Physiol.*, 149, 329-335 (1996).

Takezawa, D., Patil, S., Bhatia, A., and Poovaiah, B.W. "Calcium-dependent protein kinase genes in corn roots." *ASGSB Bull.*, 10(1), 44 (1996).

Wang, W., Takezawa, D., and Poovaiah, B.W. "A potato cDNA encoding a homologue of mammalian multidrug resistant P-glycoprotein." *Plant Mol. Biol.*, 31, 683-687 (1996).

Wang, W., Takezawa, D., Narasimhulu, S.B., Reddy, A.S.N., and Poovaiah, B.W. "A novel kinesin-like protein with a calmodulin-binding domain." *Plant Mol. Biol.*, 31, 87-100 (1996).

Mechanism of Auxin Action in Root Growth/Gravitropism

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Funding:

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Students Funded Under Research: 5

Post-Doctoral Associates: 1

FY 1997 Funding: \$122,842

Task Description:

In this research, we will use PCR-based subtractive hybridization to isolated auxin up- and down-regulated genes in tomato seedling roots. Using the auxin-insensitive tomato mutant *dgt* and other criteria, we propose to screen these cDNAs and further study those that are likely candidates for participation in IAA-mediated root growth regulation. Some of the clones will be used to generate ³⁵S antisense RNA probes for tissue print analysis of the initial phases of root gravitropism. The second part of this research describes experiments to test the hypothesis that auxin ultimately causes the down-regulation of plasma membrane H⁺-ATPase levels and/or activity. If this is the case, the asymmetric growth which causes root gravicurvature might be mediated via differential H⁺-ATPase activity and hence asymmetric H⁺ excretion. Overall, both sets of experiments (approaches) should provide a better understanding of auxin action in roots. This knowledge can then be applied to the asymmetric growth which occurs during root gravitropism allowing us to eventually validate or reject a role for auxin as the gravitropic effector.

To identify possible auxin up-and-down-regulated genes in tomato seedling roots, we employed a PCR-based subtractive hybridization procedure. We reasoned the extremely high sensitivity of this approach might allow us to isolate the relatively rare transcripts typically central to mediation of early signal transduction responses. This approach appears to have been successful, and we now have evidence for seven auxin-down-regulated messages and five auxin-up-regulated messages. These messages vary in abundance. The magnitude of auxin regulation ranges from approximately 3 to 100 fold. In the last few months, we have cloned and begun to characterize four gene fragments corresponding to these auxin-regulated messages. Once this task is complete, we will generate ³⁵S antisense RNA probes for tissue print analysis.

Our second task, analysis of H⁺-ATPase transcripts and expression in maize, is proceeding very well. We have isolated and cloned 12 genes corresponding to 12 potential ATPase isoforms. Data indicate three isoforms (MHA2, MHA3, MHA4) are expressed at moderate levels and one is expressed at low levels (MHA5) in roots. In addition, while completing experiments designed to further characterize these expressed transcripts and to obtain sequence information about their 3' untranslated ends, we made the fortuitous discovery described below.

RT-PCR of root RNA followed by TA cloning provided evidence showing cDNAs of isoform MHA4 were represented by two distinct size classes. Subsequent sequencing of these two clone classes revealed the larger

size class (MHA4) was a typical H⁺-ATPase. The smaller class of cDNAs (MHA4d), however, had a 609bp depletion near the C-terminus. The deleted nucleotides included a region known as the autoinhibitory domain.

Examination of the 3' untranslated ends (3'UTR) of MHA4 and MHA4d suggested these transcripts were derived from one gene. This conclusion was supported by the isolation and sequencing of a genomic clone. Comparison of the nucleotide sequence of MHA4 and MHA4d cDNAs with the genomic fragment indicated the latter contains four small introns; however, only intron 2 is relevant to the origin of the differences between MHA4 and MHA4d. Apparently, MHA4 is derived by simple exclusion of intron 2, while MHA4d is derived by a splice at the normal 5' intron 2-exon junction and an alternative 3' splice which is located 609 bp (of ORF) downstream from the 3' junction of intron 2.

To the best of our knowledge, there have been no previous reports of alternative splicing of plant PM H⁺-ATPases which would produce alternative C-termini. Discovery of the alternative splicing of MHA4 pre-mRNA raises many questions about relative abundance, function, and targeting of the two alternative forms. Experimental approaches to some of these questions will be one of our goals during the third year of funding.

How plants respond to gravity to produce a predictable pattern of growth is an interesting problem in developmental biology and has important ramifications regarding our ability to grow and utilize plants in the microgravity environment of space. When a plant root is placed in a horizontal position, it begins to curve downward within minutes and reestablishes its original vertical orientation within several hours. This phenomenon, known as positive gravitropism, can be divided into three components: 1) gravity perception, 2) signal transduction, and 3) asymmetric cell elongation. Since the site of gravity perception is the root apex (likely the root cap), and asymmetric cell elongation occurs several millimeters distant in the zone of elongation, some signal(s) must migrate rapidly from the cap to the elongation zone. An important problem in plant gravitation research today is the nature of this signal and how it migrates to and influences events within the zone of elongation. There is a substantial body of evidence which suggests that auxin (IAA) is this signal. However, there are also serious questions and inconsistencies which cast doubt on auxin-based models. This controversy is unlikely to be resolved until we better understand the cellular and molecular events associated with the differential regulation of cell elongation in roots caused by IAA. This area of growth physiology has been neglected and understudied relative to the mechanism by which auxin promotes the growth of shoot cells. The experiments we are conducting represent steps which may help to rectify this situation and provide new tools to test whether IAA is indeed the gravitropic effector in roots.

Cellular Bases of Light-regulated Gravity Responses

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Students Funded Under Research: 5

Post-Doctoral Associates: 1

FY 1997 Funding: \$86,854

Task Description:

The overall objective of this research is to further define and inter-relate the cellular processes that are changed under the joint influence of gravity and light to produce gravitropic growth in plants. The proposed experiments are designed to test earlier inferences that Ca^{2+} plays a regulatory role in gravitropism by trying to identify one or more of the steps in the transduction chain for gravitropic growth in which Ca^{2+} is likely to exert a critical influence. Our recent studies on a Ca^{2+} -binding protein in pea seedlings called p35 indicate that it is a member of the annexin family of proteins, and, like animal annexins, may participate importantly in the regulation of Ca^{2+} -stimulated secretion. In particular our observation that p35 is most highly concentrated in cells that are secreting wall or other extracellular matrix materials have led us to propose that annexins may play a key role in growth regulation through its function in delivering materials needed for wall construction. The experiments proposed will test this hypothesis by determining whether p35 can, like animal annexins, promote vesicle fusion and ion transport changes, whether its expression is stimulated by light signals that promote orthogravitropic growth, and whether it accumulates in curved regions where asymmetric growth is induced by the gravitropic stimulus. Structural studies to better define the similarity of p35 to known annexins will also be performed. To further test whether Ca^{2+} is a signal transducer for light and gravitropic stimuli, we will study whether these stimuli induce a change in the level or distribution of cytoplasmic free $[\text{Ca}^{2+}]$ in fern rhizoid cells.

Progress made during FY97 is summarized under two headings related to two of the three aims stated in the funded proposal. All contribute to the overall goal of attaining a better understanding of the cellular functions that are altered by gravity and light to produce gravitropic growth. Aim 1 was to determine the primary structure of annexins in plants such that specific antibody and oligonucleotide probes could be constructed to be used as tools in distinguishing the individual roles of each plant annexin. In the past year, we have obtained two different full-length *Arabidopsis* annexin cDNAs. Both cDNAs have short N-terminal sequences. Based on these two cDNA sequences and on analyses of other plant annexin sequences in the database, it is clear that plant annexins differ from animal annexins in that their N-terminal sequences are not sites for functional divergence.

Currently, we are screening an *Arabidopsis* library for annexin-binding partners using both of the annexin cDNAs in the yeast two-hybrid system. Results from these experiments should begin to elucidate the different functions of individual annexins, which is the main goal of aim 1. We have incorporated the two full-length annexin cDNAs into expression vectors and have overexpressed them in bacteria so that we can both test their biochemical characteristics and raise polyclonal antibodies to them. We are hopeful that a sub-population of the

polyclonal antibodies to each annexin will be monospecific and can be used as probes to distinguish potentially different subcellular or tissue-level distributions of these two proteins.

Using highly specific polyclonal anti-pea annexin antibodies in immunocytochemical and immunoblot assays, we have found that a subpopulation of plant annexins is localized in the nucleus. Following a procedure used for the isolation of animal primer recognition proteins (PRPs), we have found that the corresponding pea fraction contains annexin. This suggests that in plants, as has been demonstrated in animals, annexin may play a role in lagging strand DNA replication and in DNA repair.

One of the biochemical activities of the *Arabidopsis* annexins now being tested to learn more about specialization among annexins is calcium channel activity. In collaboration with a group in New Zealand, we are currently testing if this property of animal annexins is shared by plant annexins and if it is specific for one or other of the *Arabidopsis* annexins. The results from these experiments could reveal differences in function between the two annexins.

Progress has also been made in the collaborative project with Pioneer. We have obtained mutant seed for a corn root 35 kDa annexin, and have confirmed that an annexin gene in these seeds is interrupted by a Mu transposon. We will be growing the plants from this seed this winter, and will be examining them for mutant phenotypes in roots and shoots that suggest processes that require normal annexin gene function.

Aim 2 of the proposal was to examine changes in the expression and/or localization of annexins in response to gravity. We have continued to pursue preliminary data reported in FY96 that there was enhanced annexin expression in gravistimulated pea shoots. We have now confirmed that there is a gravistimulated change in localization of annexin in pea plumules. What changes is the polarity of distribution, and this change occurs within 15 minutes of the stimulus. This change appears to correlate with a change in PAS staining of cell wall polysaccharides. This is an exciting finding because it demonstrates a clear link between a calcium-binding protein and the gravitropic response.

This research does not directly seek to understand a disease or malady that affects humans on Earth and/or in space, nor does it seek to develop new therapeutics for alleviating symptoms of a malady on Earth. However, the findings will help to understand the growth of plants better, and since plants are a crucial source of food for humans, this research does seek to understand the malady of malnutrition. Also, this research does yield a new understanding of basic biological processes, specifically the processes of plant growth and the cellular mechanisms whereby gravity can affect the developmental polarity in cells. Further, this research points to a real role of gravity in regulating growth and development of plants on Earth and thus reveals potential problems in achieving normal growth and development of plants in space. Finally, the health of humans is inextricably linked to the ability to control and continuously improve the growth of plants. This, in turn, requires an improved understanding of the molecular mechanisms that control growth in plants. The accomplishments of this research contribute to that improved understanding, and thus indirectly benefit humans.

FY97 Publications, Presentations, and Other Accomplishments:

Clark, G.B. and Roux, S.J. "Biochemical characterization of nuclear pea annexins." *J. Plant Physiol.*, 114 (Supplement), 265 (1997).

Edwards, E.S. and Roux, S.J. "The influence of gravity and light on developmental polarity of single cells of *Ceratopteris richardii* gametophytes." *Biol. Bull.*, 192, 139-140 (1997).

Kim, S.-H. and Roux, S.J. "AtRanBP1C, an *Arabidopsis* Ran-binding protein, acts as a coactivator or RanGAP." *J. Plant Physiol.*, 114 (Supplement), 287 (1997).

Thomas, C.E., Gore, J.M., Naus, K.A., Lloyd, A.M., and Roux, S.J. "Evaluating roles for apyrase in plant growth and development." *J. Plant Physiol.*, 114 (Supplement), 48 (1997).

Re-Evaluation of the Role of Starch in Gravitropic Sensing

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Funding:

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Initial Funding Date: 1995

Expiration: 1998

Students Funded Under Research: 4

Post-Doctoral Associates: 1

FY 1997 Funding: \$90,301

Task Description:

The controversy about the role of starch in gravitropic sensing is old, yet we still do not know the mechanism of sensing. We have previously shown that starch-deficient mutants of *Arabidopsis* (TC7) and *Nicotiana* (NS458) are impaired in their gravitropism. While this suggests that starch is not necessary for reduced gravitropism, it also indicates that the mass of the starch contributes to sensing when present and thus is necessary for full gravitropic sensitivity. Our investigations in this area focus on three related projects: (1) the effect of light on hypocotyl gravitropism in NS458; (2) the effects (if any) of root phototropism on measurements of gravitropic sensitivity; and (3) the effects of starch overproduction on sedimentation and gravitropism.

Dark-grown hypocotyls of a starch-deficient mutant of *Nicotiana sylvestris* lack amyloplasts and plastid sedimentation, and have severely reduced gravitropism. However, gravitropism improves dramatically when NS458 seedlings are grown in the light. To determine the extent of this improvement and whether mutant hypocotyls contain sedimented amyloplasts, gravitropic sensitivity and plastid size and position were measured. We found that light-grown NS458 hypocotyls are gravitropic but reduced in sensitivity compared to the wild type. Starch occupies only a fraction of the volume of NS458 plastids in both the light and the dark, whereas wild-type plastids are essentially filled with starch in both treatments. However, light increases plastid size twice as much in the mutant as in the wild-type and these are sedimented. The induction by light of plastid sedimentation in NS458 provides new evidence for the possible role of plastid mass and sedimentation in stem gravitropic sensing.

Light interacts with root gravitropism in a different way than in hypocotyls. *Arabidopsis* roots are phototropic and we are determining if this phototropism has interfered with measurements of gravitropic sensitivity.

Finally, we have been examining the *sex1* (starch excess) mutant of *Arabidopsis* (TC265). This mutant accumulates extra starch apparently through inactivation of a hexose transporter in the plastid envelope. The presumptive gravity sensing cells of the root and stem were examined to determine whether the mutation altered gravitropism, amyloplast sedimentation, and starch content. Stereological analysis of electron micrographs of the central cap cells did not reveal any differences between *sex1* and the wild-type (WT) in plastid size, plastid number, and their proportion per cell, cell area, cell height, and relative position of plastids in the cell. Plastids in the peripheral rootcap and in the body of the root had noticeably more starch in *sex1* compared to the WT.

Both root growth and gravitropic sensitivity were comparable. In the stem endodermis, amyloplasts were about 70% larger and were sedimented over much more of the length of the stem in *sex1* compared to the WT. However, within the endodermis of a single plant, sedimenting amyloplasts from the apical region and non-sedimenting ones from the basal region did not differ in size. These data indicate that the *sex1* mutation affects different tissues differentially. However, amyloplast sedimentation is not simply a function of plastid size but is also regulated by cell-specific factors. The effects, if any, of these differences in sedimentation and plastid size on the gravitropic sensitivity of stems is currently being evaluated.

This research is in fundamental plant cell biology and does not address disease or therapeutics, nor is it likely to have any foreseeable direct impact on humans or in new technologies. It does, however, address basic biological questions of widespread interest, e.g., how do plants "know" which way is up? The long-term hypothesis that the mass of starch provides this signal requires further critical testing to establish its viability; testing is underway and is supported by the present grant. Knowledge of the basic mechanism of gravitropic sensing would be of wide biological interest, not just in the plant research community, but among all biologists and indeed with concerned citizenry including students interested in space tomatoes or in science fair projects.

FY97 Publications, Presentations, and Other Accomplishments:

Lwigale, P.Y., Denning, J., and Wiens, D.J. "Altered gravity and early heart development in culture." (Poster) American Society for Space and Gravitational Biology Annual Meeting, Charlotte, NC (1996).

Sack, F.D. "Plastids and gravitropic sensing." *Planta* In "Plant Biology in Space," 203 (Supplement), S63-S68 (1997).

Vitha, S., Yang, M., and Sack, F.D. "Restoration by light of hypocotyl gravitropism in a starch-deficient mutant of *Nicotiana* correlates with amyloplast sedimentation." (Abstract) *Plant Physiol.*, 114: (Suppl), 621 (1997).

Vitha, S., Yang, M., Bourett, T., Caspar, T., and Sack, F.D. "Gravitropism and tissue-specific phenotypes in plastid starch and sedimentation in the *Arabidopsis* *sex1* mutant." (Abstract) *Plant Physiol.*, 114 (Suppl), 1628 (1997).

Perception and Transduction of the Gravitational Stimulus

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Funding:

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Solicitation: 95-OLMSA-01

Initial Funding Date: 1996

Expiration: 1999

Students Funded Under Research: 5

Post-Doctoral Associates: 0

FY 1997 Funding: \$63,405

Task Description:

The ability to respond appropriately to the environment is essential for plant life. Gravity is a ubiquitous environmental factor that provides a cue to plants that subsequently leads to the regulation of plant form and function. Gravity either induces a polarity in cells or uses an inherent polarity to further polarize receptor cells. The gravity-induced polarity of these cells then provides the biochemical and/or biophysical conditions that allow the subsequent morphological polarities like the differentiation of the root/shoot axis or gravitropism of plant parts to take place.

In this research, we present experiments that are aimed at elucidating a mechanism of gravisensing used by statolith-free cells by continuing our investigations on the gravity-induced polarity of cytoplasmic streaming in *Characean* internodal cells. In order to better understand the mechanism of gravisensing, we will use gel electrophoresis combined with Western blotting, affinity chromatography, and immunolocalization in order to determine the identity of the putative gravireceptor and its localization. We will also continue our work on determining how the vectorial energy inherent in the gravitational field is transduced into a biophysical signal that the cell uses in order to initiate the cellular activities that lead to the graviresponse. In order to accomplish this goal, we will measure gravity-induced changes in the Ca^{2+} fluxes using Sr^{2+} as a tracer as well as changes in the intracellular free $[\text{Ca}^{2+}]$ using Fura-2-tagged dextrans after artificially inducing the graviresponse using unidirectionally applied-hydrostatic pressure.

The fact that we are using single cells to study a graviresponse in plants means that we can exploit the cell biological tools and principles that have been developed to study stimulus perception and signal transduction. However, we believe that our conclusions can be generalized to explain gravisensing in higher plants, fungi, and animals (i.e., the sense of balance).

We have proposed a model for gravisensing in which the entire protoplast experiences the force of gravity and settles within the extracellular matrix. Consequently, integrin-like proteins connecting the plasma membrane to the extracellular matrix at the ends of the cell experience a differential compression or tension as a result of gravitational pressure. We have shown that the cell does not sense the vector of gravity directly, but the tension and compression that results from the static buoyancy of the protoplast relative to the external medium within

the extracellular matrix. The tension and compression at the plasma membrane-extracellular matrix junction, thus induced, leads to an activation of certain classes of Ca^{2+} channels, localized at the ends of the cell. The increased activation at the site of tension results in a polarity in the flux of Ca^{2+} . The consequence of inducing a polarity in the flux of Ca^{2+} is that a polarity in the velocity of cytoplasmic streaming results because the velocity away from the site of the higher flux increases while the velocity away from the site of the lower flux decreases.

We have demonstrated that *Chara* cells do not sense the gravity vector directly, but sense gravity indirectly by sensing the tensile and compressive forces that result from the sinking of the protoplast within the fluid of the extracellular matrix. We have also found specific inhibitors of the tension and compression receptor(s). The oligopeptide RGDS inhibits gravity sensing when it is applied to the top of the cell, when the density of the protoplast is greater than the density of the external medium or when it is applied to the bottom of the cell when the density of the protoplast is less than the density of the external medium. This means that RGDS inhibits gravity sensing when and only when it is applied to the end of the cell that experiences tension.

We have also found that the oligopeptide YIGSR inhibits gravity sensing when it is applied to the bottom of the cell, when the density of the protoplast is greater than the density of the external medium, or when it is applied to the top of the cell when the density of the protoplast is less than the density of the external medium. This means that YIGSR inhibits gravity sensing when and only when it is applied to the end of the cell that experiences compression.

Ca^{2+} is normally required for the graviresponse in *Chara*. We have found that Sr^{2+} can substitute for Ca^{2+} in the gravity response. Using Sr^{2+} as a tracer for Ca^{2+} , we have found that the flux of Sr^{2+} increases from $20 \text{ nmol m}^{-2} \text{ s}^{-1}$ to $60 \text{ nmol m}^{-2} \text{ s}^{-1}$ at the end of the cell that experiences tension. The increased flux of Sr^{2+} is inhibited by RGDS. The increased activation at the site of tension results in a polarity in the flux of Sr^{2+} . The consequence of inducing a polarity in the flux of Ca^{2+} is that a polarity in the velocity of cytoplasmic results because the velocity away from the site of the higher flux increases while the velocity away from the site of the lower flux decreases. We are continuing to characterize the factors involved in the relationship between the activation of the tension and compression receptors and the flux of Sr^{2+} . For example, we have found that the microtubule-depolymerizing agent APM inhibits both the gravity-induced polarity in the Sr^{2+} flux as well as the gravity-induced polarity of cytoplasmic streaming.

In the course of our experiments, we have found that the ability of cells to sense gravity depends on the external Ca^{2+} concentration. However, we have also found that the membrane potential and conductance are also influenced by the external Ca^{2+} concentration in an identical manner. Therefore, we constructed and put into operation a voltage-clamp amplifier to directly test the effect of external Ca^{2+} on various membrane transporters. Using voltage clamping, we hope to identify ion transporters that may be important in gravisensing. We also hope that we can identify and control for any secondary effects that may result from the manipulation of external Ca^{2+} .

In order to determine how Ca^{2+} influences the polarity of cytoplasmic streaming, we are currently isolating myosin from *Chara* using an *in vitro* motility assay. Once we have successfully isolated myosin, we will see how Ca^{2+} and other cellular substances (e.g., calcium-dependent protein kinases) affect its ability to interact with actin.

While we are continuing to deepen our understanding of gravity sensing in *Chara*, we are also broadening our scope and testing our model of gravity sensing in higher plants and fungi. We are studying the positive gravitropic responses of rice roots. We have shown that varying the density of the external medium affects the gravitropic response of roots in a manner similar to how it affects the graviresponse in *Chara*. We have also tested the role of actin in gravitropism of roots. By applying cytochalasin D to roots and measuring their gravitropic curvature as well as visualizing the actin in the cells with fluorescence microscopy, we have determined that actin is not required for gravitropism as required by the statolith-actin filament hypothesis of

gravisensing. These data suggest that the gravitational pressure model for gravisensing that came out of our work with *Chara* may also apply to higher plants.

We have tested the ability of the plasmodial stage of the slime mold *Physarum polycephalum* to sense gravity. Unfortunately we are unable to reproduce the gravitaxis response seen by others. *Physarum polycephalum* also has an amoeboid and a flagellated stage in its life cycle. The former bases its motility on the actin cytoskeleton while the latter bases its motility on the tubulin cytoskeleton. Cells in solution become flagellated while cells on a solid surface become amoeboid, suggesting that the cells are capable of sensing their own weight. In order to test this hypothesis, we subjected the cells to various centrifugal forces and found that cells in solution tend to become amoeboid under higher and higher centrifugal forces, and this effect is reversed by increasing the density of the external medium. These results suggest that the gravireceptors of *Physarum*, like those in *Chara*, are localized at the plasma membrane-extracellular matrix junction, and moreover that the gravitational pressure model may also apply to fungi.

Our work has contributed to understanding the importance of the extracellular matrix in influencing physiology and development. It has also contributed to understanding the importance of calcium in signal transduction pathways and in the development of polarity. In terms of medicine, understanding the mechanism of gravisensing at the cellular level may contribute to understanding and treating the problem of loss of balance that is experienced by elderly people.

FY97 Publications, Presentations, and Other Accomplishments:

Staves, M.P., Wayne, R., and Leopold, A.C. "Cytochalasin D does not inhibit gravitropism in roots." *Amer. J. Bot.* (In Press).

Staves, M.P., Wayne, R., and Leopold, A.C. "The effect of the external medium on gravitropic curvature of rice (*Oryza sativa*) roots." *Amer. J. Bot.* (In Press).

Staves, M.P., Wayne, R., and Leopold, A.C. "The effect of the external medium on the gravity-induced polarity of cytoplasmic streaming in *Chara*." *Amer. J. Bot.* (In Press).

Wayne, R. and Staves, M. P. "A down to Earth model of gravisensing or Newton's Law of Gravitation from the apple's perspective." *Physiol. Plant*, 98, 917-921 (1996).

Wayne, R. and Staves, M. P. "A down-to-Earth model of gravisensing." *ASGSB Bull.*, 10, 57-64 (1997).

Wayne, R. and Staves, M. P. "The August Krogh Principle applies to plants." *BioScience*, 46, 365-369 (1996).

Remote Sensing for Research and Control of Malaria in Belize

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Kevin O. Pope, Ph.D.; Geo Eco Arc Research, La Canada, CA
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Funding:

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Initial Funding Date: 1995	Expiration: 1998
Students Funded Under Research: 5	Post-Doctoral Associates: 1
FY 1997 Funding: \$393,214	
Joint Agency Participation: DoD (USUHS)	

Task Description:

A three-year program of research is proposed to address specific science issues leading to the application of remote sensing (RS) and geographic information system (GIS) technologies to target and manage malaria vector (*Anopheles* mosquitoes) control in Belize. This project is a natural extension of NASA's project to develop predictive models, driven by satellite data, of malaria transmission potential. This is a subject of increasing interest and has been the topic of recent science news articles. Malaria was selected for study because of its global importance and a predictive capability could lead to improved, cost-effective malaria control. It is proposed to use multispectral satellite data to predict disease (malaria) trouble spots based on clear understandings of environmental factors that determine the presence of disease vectors. This will be a multidisciplinary program of research involving multiple organizations with Belize as the performance site. Belize is characterized as a small country with a "big" malaria problem. Research activities include such diverse efforts as field and laboratory studies, use of remote sensing and geographic information system technologies, mathematical modeling, developing predictions and testing new technologies, as well as training and capacity building. The hypothesis is: Remote sensing and geographic information system technologies, employed within a paradigm of systematic field and laboratory studies, can be developed as tools to cost-effectively target and prioritize the application of vector control measures within a national malaria control program. The results of our research has allowed us to focus efforts on only three important vector species in Belize. Predictive capabilities have been developed, using remote sensing data, for the 3 important malaria vector species. Predictions are presently being tested against malaria data for multiple population centers throughout Belize. Additionally, we are now attempting to develop a two-track program of operations and research to more fully implement the successes of this NASA-sponsored research effort within the national malaria control program of Belize.

This research effort has made significant progress in meeting projected research goals. Objectives for the 12-month period were defined during the investigator's workshop in June 1996. In brief, we fully characterized the environmental requirements of *Anopheles vestitipennis* and we have used these defined relationships and

remote sensing data to detect favorable sites for the presence and abundance of this important vector species. By using our enzyme-linked immunosorbent assay capabilities, we also quantified the roles and importance of the four vector species present in Belize. We now know that only three species are of significant medical importance. A mathematical model that can be used to define thresholds of vector abundance has been developed. This model is being used in conjunction with our predictive capabilities to discriminate between high, moderate, and low-risk sites for transmission by the three important vectors in Belize. The spatial distribution of malaria cases in population centers in Belize have been studied and the analyses support a new conceptual model for malaria control. We propose use of GIS and remote sensing data, in combination with historical data on malaria cases, to more cost-effectively manage scarce malaria control resources in Belize. Costing data are being compiled to show that the new approach to malaria control can be carried out cost-effectively. The data include costs for malaria control operations, remote sensing and GIS capabilities, and personnel. Preliminary analyses show that the savings in malaria control operations, in combination with decreased costs associated with fewer cases of malaria, should offset the cost of the new technologies in a national malaria control program. Consensus of participants in the 1997 workshop in Belize was that we should attempt to develop a two-track approach to implementing the successes of the NASA-sponsored research in Belize. One track would be to obtain funding for fully implementing the conceptual malaria control model within the National Malaria Control Program in Belize. This would comprise the operational track. The second track will be to continue research to address questions being raised by the current research program. The latter would comprise the research track and it would target basic science issues in the use of remote sensing in malaria control.

Malaria is the preeminent reemerging disease in the Americas. The application of remote sensing technology to the study of this disease and its mosquito vectors is providing new and critical information for the proper management of malaria in developing countries. This research definitely seeks to understand the dynamics of a human disease on Earth. The research goal is to test the applicability of predictive models based on the use of multispectral satellite data to target applications of malaria control measures. Successful, cost-effective applications of remote sensing technology to the Belize National Malaria Control Program will have broad implications for malaria control throughout the world. This research has already resulted in a critical revision of our understanding of malaria epidemiology in Belize. As background, when we initiated research, the only recognized vector of malaria in Belize was *Anopheles albimanus*. Historically, all surveys and studies focused entirely on this vector species. However, our broad-based program of research has shown that at three species are truly important vectors of human malaria. We can now characterize these vectors by specific environments and seasons and environmental surrogates can be employed, in combination with remote sensing data, to predict the time and location of malaria risk conditions. Thus, we are showing that satellite data can be used to predict where and when humans are at risk of malaria transmission. In the last 12 months, we have developed a new and innovative conceptual model for use of remote sensing and GIS technologies in combination with the national malaria control data base to cost-effectively control malaria in Belize. This new model will allow us to carefully target houses for insecticide spraying, which will not only reduce the total amount of malaria within the human population, but will also reduce the total amount of insecticide used for malaria control. We are hopeful that this new model will serve to revitalize malaria control efforts in many countries of the world.

FY97 Publications, Presentations, and Other Accomplishments:

Achee, N., Hakre, S., and Greico, J. "The use of enzyme-linked immunosorbent assays (ELISAs) to evaluate infection rates in malaria vectors in Belize, C.A." Presented at the USUHS Research Day 1997 at USUHS, Bethesda, MD (March 24, 1997).

Roberts, D.R. "Applications of remote sensing and GIS technologies for malaria control in Belize, C.A." Presented at the Symposium "Infectious Diseases and Tropical Medicine at the USUHS Research Day 1997 at USUHS, Bethesda, MD (March 25, 1997).

Roberts, D.R., Rejmankova, E., Andre, R.G., Paris, J., and Pope, K. "The use of GIS and remote sensing technologies in the national malaria control program in Belize." Presented at the National Environmental Health Associations 61st Annual Education Conference and Exhibition, Arlington, VA (July 1, 1997).

Gender Differences in the Responses of Rhesus Monkeys to a Hyperdynamic Environment

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Funding:

UPN/Project Identification: 199-99-27-08	Solicitation: GSRP 95
Initial Funding Date: 1995	Expiration: 1998
FY 1997 Funding: \$22,000	

Task Description:

This research examines the influences of gravity and light on the circadian system (CTS) in unrestrained mature male and female rhesus macaques (*Macaca mulatta*). The CTS coordinates the temporal aspects of physiology and behavior. The light-dark cycle is the major time cue used by the CTS. Disruptions in circadian timing adversely affect an organism's ability to respond to environmental challenges, decrease performance and contribute to psychological disorders. Circadian timing is altered under both the microgravity of space flight and hyperdynamic fields produced by centrifugation. Our experiments will determine the effects of a hyperdynamic environment on the CTS in rhesus monkeys and characterize any gender differences in CTS function.

This research program has made significant progress in its examination of the effect of light on circadian rhythms in rhesus macaques. We have successfully trained both male and female rhesus monkeys in the use of the Psychomotor Test System (PTS), developed at the University of Georgia Language Research Center. We have shown that rhesus have rhythms in performance, and that we can measure these along with other physiological variables. In the first study, baseline data were collected under LD 16:8 for two weeks, then the animals were exposed to constant light (LL) for a period of 6 weeks. During the first two weeks, under the LD cycle, the performance rhythm maintained a strict phase relationship with the 24 hour cycle. With the onset of LL, the drinking pattern revealed that the internal clock of this animal has an endogenous period that is slightly less than 24 hours. The mean period of performance in LL was 23.6 hours. There was no significant difference in the mean number of PTS trials per day between LD and LL, but the amplitude of the performance rhythm was significantly smaller in LL. The second study examined the response of the circadian system of the rhesus monkey to a phase shift in the light-dark cycle. Such a phase shift results in a transient internal desynchronization of rhythms, analogous to the effects of jet-lag in humans. It was shown that drinking and performance respond similarly to phase advances and delays. In addition, we completed a 2-G pilot study in male rhesus monkeys showing an initial suppression of rhythmicity, followed by a gradual return of circadian function. We have also tracked the cyclicity of eight female rhesus for the past year. Daily urine samples have been collected and will be assayed for estrone and progesterone conjugates to confirm the cycling status. Once this is confirmed, we will begin using the females in our 1-G and 2-G studies.

We know from previous research that exposure to space flight affects the circadian rhythms of organisms ranging from unicells to primates. Deficits in CTS function are associated with various disorders in humans, such as jet-lag, problems associated with shift work, and delayed sleep phase insomnia, and have a high co-morbidity with various mental disorders. Desynchronization between internal rhythms may be linked to reduced

capabilities in the performance of simple tasks and to psychological abnormalities. Winter depression, also known as seasonal affective disorder, has been associated with circadian rhythm desynchrony and successfully treated with early morning bright artificial light exposure.

There is a preponderance of women among those treated for psychological disorders, including those linked to circadian dysfunction. This has been attributed to various physiological, psychological, and sociological differences, but no innate underlying cause has yet been proved. Since women now form a substantial part of the space research program and are frequent space travelers, it is important to include both genders in this research.

This research will further our understanding of circadian rhythm desynchrony and the gender differences involved.

FY97 Publications, Presentations, and Other Accomplishments:

Barger, L.K., Hoban-Higgins, T.M., and Fuller, C.A. "Effects of lighting conditions on circadian rhythms of performance and drinking in rhesus monkeys." *FASEB J.*, Vol 11 (3), 3667 (1997).

High Performance Polymers for Cation Separation and Detection in Aqueous Environments

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Initial Funding Date: 1995

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

The online detection of inorganic cations in aqueous environments has become increasingly important in wastewater monitoring and water purification testing. The proposed research involves the development of a polymeric material that has ion exchange properties and may act as a sensor that induces a change in UV-vis absorption upon interaction, therefore allowing simple separation and detection of the cations. The polymers will be formed and studied in a Langmuir environment, which results in highly oriented pendant groups and a more microstructurally regular polymer. Structural order may enhance sensitivity and response time in this application.

The recent focus of this project has been concentrated in two areas: first, the polymerization of a simple monomer in a Langmuir environment, and second, the synthesis of the complex monomer 2-(2,5-bis (bromomethyl) phenyl)-4,5-bis (4,4' methoxy phenyl) imidazole.

We have successfully polymerized octadecyl (2,5-bis (tetrahydrothiopheniumylmethyl)) benzoate dibromide to form a functionalized polyphenylenevinylene (PPV) in a Langmuir environment. The polymer was studied and was found to produce stable monolayers over time. The polymers were collected and characterized by conventional techniques. Gel permeation chromatography indicated that the polymer had a molecular weight of 13,000 and fluorescence data showed a maximum intensity at 430 nm.

The synthetic route to 2-(2,5-bis (bromomethyl) phenyl)-4,5-bis (4,4' methoxy phenyl) imidazole has been developed after many attempts to find a route in which each of the steps produces sufficient yields. Each of the eight steps of the convergent route that we have developed has yields of 70% or more. In addition, a water soluble model compound has also been synthesized and was used to successfully complex a number of cations including copper, magnesium, calcium, and cobalt.

The positive results of this year will now be brought together to test the possibility of using imidazole functionalized PPV as a sensor for specific cations.

NASA's interest in sensors stems from the need for rapid, online detection of plant nutrients such as ammonium, calcium, potassium, and magnesium as well as plant toxins like sodium. The detection of these cations has become increasingly important to crop maintenance as part of bioregenerative life support systems in the Biomass production chamber at Kennedy Space Center. The application of this type of sensor could be

extended to wastewater monitoring and water purification testing, which may be especially useful for hydroponic plant growth systems and farm type fisheries.

FY97 Publications, Presentations, and Other Accomplishments:

Duran, R.S. "Characterization of functionalized monolayer films." Presented at University of Pierre and Marie Curie, Paris, France (January 22, 1997).

Duran, R.S. "Characterization of functionalized monolayers." Presented at University of DeMaine, LeMans, France (January 23, 1997).

Duran, R.S. "Characterization of functionalized monolayers." Presented at University of Montpellier, Montpellier, France (January 27, 1997).

Duran, R.S. "Squeezing on monolayers, what can we learn?" Presented at CNRS, Materials Research Institute, Strausburg, France (June 3, 1997).

Differential Gene Expression in Germinating Spores of Ceratopteris richardii During the Period of Responsiveness to Gravity

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Solicitation: not available

Initial Funding Date: 1996

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FY 1997 Funding: \$22,000

Task Description:

Gravity affects certain aspects of early polar development in germinating spores of a fern, *Ceratopteris richardii*. The gravity directed event is the downward migration of the spore cell nucleus from a central position to a lower position. This migration is a crucial developmental event. Though it is certain that gravity affects early developmental events, the mechanism by which it does so remains uncertain. We are interested in establishing the molecular and genetic basis for this response. The overall strategy will be to establish the period in which the spores are responding to gravity; freeze spores before, during, and after this period; extract their total RNA; evaluate differential gene expression by both Differential Display RT-PCR and Northern analysis at the time points sampled; identify and characterize any genes that are differentially expressed; and overexpress the antisense sequence in an attempt to "knockout" the expression of the gene in sporophytes of the fern. Preliminary results are indicative of differential gene expression. We have identified eighteen partial cDNAs using DDRT-PCR. Of 6 that have been used as probes against the total RNA, we have verified 5 as being truly differentially expressed by Northern Analysis and have cloned and sequenced them. Since Differential Display yields partial cDNAs, we are performing further analyses to obtain the full length sequence. Thus far by 5' RACE, we have obtained 1200bp of sequence for 2 probes. Concurrently, a cDNA library that we have constructed from *Ceratopteris* is also being screened. Results expected from the experiments proposed will identify and characterize genes that are potentially involved in conferring graviresponsiveness in germinating spores of *Ceratopteris richardii*. This would be a truly unique contribution and a critical first step toward demonstrating the genetic components of a gravity response. Further investigation should assist in gaining a clearer understanding of the molecular and genetic basis behind gravity responses in early polar development in plants.

This project is in its early stages of funding. Progress is based on work towards the accomplishment of the following objectives: (1) to obtain an initial estimate of the number of genes that are differentially expressed during the window of polarity fixation, when spores of *Ceratopteris richardii* are responding to gravity; (2) to identify and characterize genes that are differentially expressed during the window of polarity fixation; and (3) to evaluate the relative importance of the differentially expressed genes to the gravity response by seeing if the response is affected or inhibited when the expression of these genes is diminished.

Progress towards Objective 1 is as follows: The method of differential display reverse transcriptase-PCR (DDRT-PCR) was used to determine the number of genes that are differentially expressed during the window of

polarity fixation. Total RNA was extracted from 4 timepoints along the period of graviresponsiveness and used in DDRT-PCR. Over 20 candidate cDNAs have been identified to be differentially expressed using only 1 primer combination of the possible 96 combinations that would encompass the entire genome.

Progress towards Objective 2 is as follows: Candidate cDNAs found to be differentially expressed were excised from the Differential Display gel, eluted, and reamplified. 7 of these candidates were subcloned. Northern analysis was performed on 7 of the partial cDNAs. They were all found to be upregulated in the range of 1.5 to 2.5 Kb. A cDNA library was constructed from gametophytes of *Ceratopteris*. This library is currently being screened with probes obtained from DDRT-PCR to obtain full length sequences. The method of 5' RACE is also being employed to obtain additional sequence information on the probes obtained by DDRT-PCR. For one of these cDNAs, 700bp of sequence was obtained by this method in addition to the original 456bp fragment for a total of 1156 bp of sequence. To verify that the two cDNAs are indeed from the same sequence, Northern analysis was performed and observed for hybridization to the same location and identical size gene. Both cDNAs showed identical hybridization patterns and size and both showed a dramatic upregulation at approximately 42 hrs after germination during which 85% of the spores are responding to gravity. There was a disappearance of this gene product at 66 hours after germination in which 89% responded to gravity. These cDNAs show a gene product at 5.0 kb. This does not represent the entire sequence, so 5'RACE is being performed again on the upstream region of the 1200 bp fragment. An additional 1000bp of sequence was obtained by 5'RACE as shown by Southern analysis for another probe that was originally 179bp. This is currently being cloned and sequenced.

Progress toward Objective 3 is as follows: To evaluate the relative importance of the differentially expressed genes to the gravity response, experiments are being conducted to introduce antisense constructs of the differentially expressed probes into *Ceratopteris* in an effort to "knockout" the expression of these genes and to observe the phenotypic change that results from this.

This research is directed toward obtaining a better understanding of how gravity affects basic biological processes such as plant growth and development on Earth. The focus is on understanding the cellular and molecular basis for gravity responses in a single cell plant system, *Ceratopteris richardii*. Many of the mechanisms by which gravity acts still remain a mystery. Unraveling the molecular and cellular basis behind the effects of gravity on plants will not only aid in gaining a general understanding of how gravity influences aspects of plant biology but also expand the realm of knowledge necessary for using plants to help human exploration of the space frontier.

FY97 Publications, Presentations, and Other Accomplishments:

Chatterjee, A. "Gene expression due to a gravity response in ferns." Presentation at American Society of Plant Physiologists, Vancouver, BC (August, 1997).

Chatterjee, A., Edwards, E., and Roux, S.J. "Differential gene expression in germinating spores of *Ceratopteris richardii* during the period of responsiveness to gravity." ASGSB Bull., 10, 31 (1996).

Determining Lymphocyte Responsiveness to Low Gravity Environments

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Funding:

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Solicitation: not available

Initial Funding Date: 1997

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FY 1997 Funding: \$22,000

Task Description:

The purpose of this project is to study the responses of human lymphocytes exposed to altered gravity and launch stresses, specifically the influence on cell growth, differentiation, and apoptosis. Investigations will include evaluations of gene regulation, expression of cell surface receptors, and cytoskeletal structures.

The study is in its early stages. Thus far, we have noted an increased number of apoptotic cells in the flight samples.

Understanding the mechanisms of immunity will help in the development of new treatments to combat disease.

Computer Simulation of Cardiovascular Function in Reduced Gravity

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Funding:

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Solicitation: GSRP 95

Initial Funding Date: 1995

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

Space travel poses physiological challenges to humans due to the change from terrestrial gravity to microgravity, followed by return to terrestrial gravity. The cardiovascular system undergoes a series of functional adaptations and deconditioning, resulting in orthostatic intolerance which can have an adverse effect on crew function during reentry into the Earth's gravitational field. Lower body negative pressure (LBNP) is being used to investigate cardiovascular control, and orthostatic intolerance due to increased gravitational forces. Dietary manipulations are being employed and the effect on orthostatic tolerance is under examination.

A study was undertaken to determine the gender effect on blood volume shift out of the chest during lower body negative pressure (LBNP) up to -40 mm Hg. Measurements were conducted in a population of 5 men and 5 women. Women had smaller pulmonary capillary blood volumes and diffusion capacity of the lung (DLCO) than men at 0 mm Hg. This difference remained throughout the entire LBNP range. A slide presentation of this study was made at the 68th Annual Scientific Meeting of the Aerospace Medical Association in May, 1997. A publication entitled "Pulmonary Capillary Blood Volume During Lower Body Negative Pressure: Effect of Gender" has been accepted for publication in *Aviation, Space and Environmental Medicine*.

Examination of cardiovascular and hemodynamic responses to presyncopal levels of LBNP in men is underway. Data collection has been completed and blood samples have been analyzed for catecholamines and plasma renin activity. Echocardiography was employed to measure cardiac dimensions and volumes, and to calculate ejection fraction as an index of cardiac contractility. A repeated measures design was implemented with subjects being tested once on a "normal" diet and once after a five-day sodium-restricted diet. Initial diets were randomized and sodium intake was verified by urinalysis of two 24-hour urine collections for each diet period. Analysis of study results is underway.

New questions arising from the work completed are: Is the cardiovascular control during LBNP similar between men and women on a sodium-restricted diet? What is the optimal level of sodium restriction? When data collected from this study are incorporated into the Melchior mathematical model and computer simulation of cardiovascular response to LBNP, will the model be able to predict presyncope?

The goal of this research is to examine the cardiovascular mechanisms responsible for orthostatic-induced syncope. Orthostatic intolerance, or the inability to withstand the upright position, is experienced in 64% of astronauts returning from microgravity and could pose serious risks during the re-entry and landing phases of the

space shuttle. On Earth, the situation of bedrest causes physiological changes that are similar to those experienced during space flight and predispose the patient to orthostatic intolerance, thus complicating and confounding the original malady that necessitated bedrest. The present investigation is aimed at identifying the mechanism(s) that causes orthostatic-induced syncope. The simple manipulation of changes in dietary sodium to improve orthostatic tolerance is being examined.

FY97 Publications, Presentations, and Other Accomplishments:

Davrath, L.R., Gotshall, R.W., and Tucker, A. "Changes in pulmonary blood volume and diffusion capacity with lower body negative pressure." (Abstract) *Aviat. Space Environ. Med.*, Vol 68 (7), 269 (1997).

Davrath, L.R., Gotshall, R.W., and Tucker, A. "Changes in pulmonary blood volume and diffusion capacity with lower body negative pressure." Presented at the 68th Annual Scientific Meeting of the Aerospace Medical Association (May, 1997).

Gotshall, R.W. and Davrath, L.R. "Pulmonary capillary blood volume during lower body negative pressure: Effect of gender." *Aviat. Space Environ. Med.* (In Press).

The Biomechanics of Reduced Gravity Locomotion

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UPN/Project Identification: 199-99-27-06

Solicitation: GSRP 95

Initial Funding Date: 1995

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

The general purpose of this research is to examine the mechanics and control of legged locomotion with regard to the demands of space environments. Gravity levels lower than 1.0 G represent a unique challenge to humans and other animals because gravity dependent forces are a key component to the dynamics of locomotion. When legged animals walk, they depend on an exchange of gravitational potential energy and kinetic energy with each step in order to reduce the mechanical work of locomotion. These center of mass energy fluctuations are similar to those demonstrated by a pendulum and have led to a simple model of an inverted pendulum for walking. When legged animals hop or run, gravitational potential energy and kinetic energy are in phase with each other so that no significant exchange occurs between them. However, hopping and running animals store and return elastic energy in their muscles, tendons, and ligaments with each step so that their overall mechanics resemble a simple spring-mass system. The dynamics of the system depend on gravity during the aerial phase, and depend on gravity, mass, and leg stiffness during ground contact. Our primary goal is to examine how these basic locomotion dynamics change at reduced gravity levels. In addition, because the fastest and most agile legged robots display spring-mass mechanics similar to humans, a secondary goal is to examine how humans adjust to accommodate different surface properties. The control strategies gleaned from these studies should facilitate the design and construction of legged robots capable of traversing moon and Mars terrains.

In order to study locomotion at hypo-gravity levels, we built a reduced gravity simulator that uses a series of rubber springs connected to a support harness. Stretching the springs with a winch provides a nearly constant upward force on the subject's center of mass as they walk or run on a treadmill. A force transducer in series with the springs allows us to measure the level of simulated gravity.

Our first project examined the effect of simulated reduced gravity on the preferred walk-run transition speed. Based on the inverted pendulum model for walking, we hypothesized that the transition speed would decrease with gravity level so that humans would switch from a walk to a run at slower speeds. In addition, we hypothesized that the transition speed would occur at about the same Froude number (i.e., ratio of centripetal and gravitational forces: v^2/gL , where v is the forward velocity, L is the leg length, and g is the acceleration due to gravity) for different gravity levels. The results supported our hypotheses and revealed that the Froude number corresponding to the transition speed was about 0.5 for all gravity levels. Findings from this and other studies suggest that humans will often prefer to run or even hop (as demonstrated by the Apollo astronauts) in reduced gravity instead of walk.

Given the results from the first study, our next project focused on how reduced gravity might affect the spring-mass mechanics of hopping. Although increased mass (e.g., a space suit) and reduced gravity will both alter body weight, changes in mass and gravity have fundamentally different effects on the mechanics of a spring-mass system. The resonant frequency of the system depends on mass and stiffness, but not gravity. We hypothesized that humans would adjust their leg stiffness for added mass but would maintain the same leg stiffness in reduced gravity. Although we found that leg stiffness did increase with added mass, surprisingly it also increased with reduced gravity. Interestingly, for both conditions there was a single linear relationship between body weight and Groucho number (i.e., a dimensionless measure of the “hardness” of the interaction between subject and surface). Because the peak ground reaction force in body weights is a function of the Groucho number, the peak ground reaction force was not proportional to body weight. At the lowest gravity, it was nearly 3.5 times body weight, and at the greatest body mass it was only about 2 times body weight. In addition, the increased leg stiffness for both decreased gravity and added mass strongly suggests that adding mass to submerged humans is not an accurate method for examining the mechanics of bouncing gaits.

Most recently, we have begun to investigate muscular activity and reflex modulation when humans locomote at different gravity levels. Past studies that have examined the metabolic cost of locomotion in simulated reduced gravity have found that the energetic cost of running decreases in direct proportion with gravity level, but that the energetic cost of walking does not display a one to one proportional change with gravity level. Based on these results, we have hypothesized that the muscular activity, as measured by electromyography, would decrease in direct proportion to gravity during running, but would not decrease as much for walking. We have collected data on eight subjects for this study but have not yet analyzed this data. Furthermore, to investigate how the central nervous system adapts to reduced gravity levels, we have measured the amplitude of the Hoffman reflex (i.e., H-reflex) for the soleus muscle during walking and running in reduced gravity. We have hypothesized that the H-reflex will decrease in parallel with the gravity level for both walking and running. We have collected data on seven subjects for this study, but have not yet analyzed the data.

To provide insight into possible control strategies for legged robots on varied terrain, we have investigated how humans adjust their leg stiffness to accommodate different surface properties. A compliant surface would change the dynamics of bouncing gaits if it increases the displacement of the runner's center of mass during ground contact. We hypothesized that humans would counteract the displacement of the surface by increasing their leg stiffness on compliant surfaces. We found that when humans hopped in place, their adjustments in leg stiffness maintain similar center of mass dynamics across a 1,000-fold change in surface stiffness. We have recently extended these findings to include forward running on viscoelastic surfaces. Similar to hopping, human runners adjust their leg stiffness to accommodate different running surfaces and keep many running parameters (e.g., ground contact time, peak ground reaction force, stride frequency, etc.) independent of surface stiffness. Incorporating an adjustable leg stiffness with the proper control algorithm would also allow robots to maintain similar running dynamics on different surfaces and may make it much easier to achieve dynamic stability on varied terrain. We are currently examining how humans adjust to uneven surfaces and abrupt changes in surface properties as this is likely to be the case for robots on natural terrain.

These projects are designed to study the basic principles which govern legged locomotion and thus, the results have important implications for understanding both normal and pathological gait in humans. The potential applications of this research include the determination of effective exercise countermeasures for both astronauts and obese populations, projections of astronaut capabilities and space suit designs for planetary surface EVAs, the construction of prostheses and legged robots capable of traversing a variety of terrain, and the diagnosis and prevention of locomotion-related injuries on natural and artificial surfaces.

FY97 Publications, Presentations, and Other Accomplishments:

Farley, C.T. and Ferris, D.P. "The biomechanics of walking and running: From center of mass mechanics to muscle-tendon action." *Exerc. Sport Sci. Rev.* (In Press).

Ferris, D.P. and Farley, C.T. "Interaction of leg stiffness and surface stiffness during human hopping." *J. Appl. Physiol.*, 82, 15-22 (1997).

Ferris, D.P. and Farley, C.T. "Leg stiffness adjustments during hopping and running." (Abstract) Program and Abstracts for the Seventh Annual Meeting of the Neural Control of Movement Society C-11, 23 (1997).

Ferris, D.P., Louie, M., and Farley, C.T. "Adjustments in running mechanics to accommodate different surface stiffnesses." *Physiol.*, 39, A59 (1996).

Kram, R., Domingo, A., and Ferris, D.P. "Effect of reduced gravity on the preferred walk-run transition speed." *J. Exp. Biol.*, 200, 821-826 (1997).

Cardiovascular Response to Gravitational Acceleration Using a Multi-Element Hydraulic Mock Circulation System

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Funding:

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FY 1997 Funding: \$22,000	

Task Description:

The objective of this project is to better the understanding of the physical mechanisms prompting responses by the cardiovascular system to the weightlessness of space flight by measuring the effects of microgravity and hypergravity on the human cardiovascular system using a three-element mock circulation system. The focus of the research will be on the three effects of gravitational acceleration: 1) gravity influence on ventricular filling, 2) pericardial pressure, and 3) blood fluid volume shifts throughout the body.

A three-region hydraulic model of the human systemic circulation was designed and built. This model was then flown aboard the KC-135A turbojet transport operated by the Reduced-Gravity Program of the NASA Lyndon B. Johnson Space Center (JSC) in Houston, Texas. The KC-135A flies parabolic arcs to produce weightless periods of about 25 seconds. In addition to the weightless periods, the KC-135A also experiences hypergravity of approximately 1.8-G during each parabola. Ten parabolas are flown in succession and then a short period of 1-G is attained which allows for reconfiguration of the experiment, namely the change of static systemic fluid volume. The hydraulic model incorporated an artificial left ventricle, a caudal venous pool and three vascular sections of a mock circulation system (MCS) - one for the head and arms (Cranial MCS), one for the torso (Central MCS), and one for the legs and feet (Caudal MCS) - to allow investigation of the fluid shifting in the vasculature, and changes in the cardiac performance due to gravity. The model was flown in three postures: supine, upright, and shuttle launch (legs elevated 40 cm above the heart).

After flying the model in September of 1996, it was determined that while the present design worked under 1g conditions, under changing gravitational conditions the volumetric flow throughout the regions became unstable; while the total aortic outflow remained constant, the regional flows could not be maintained in a physiologically correct ratio. To eliminate this instability, a new system was to be designed. In addition, a Pulmonary regional MCS would be added, as well as an artificial right ventricle creating a complete cardiovascular system. The prototype of the new regional MCS would be tested on board the KC-135A in April 1997. The new human cardiovascular model would be built and flown on the KC-135A in October 1997.

The three-region hydraulic model of the human systemic circulation was flown onboard the NASA KC-135A aircraft during the week of September 8, 1996. The model consisted of several components. Each MCS had four elements - proximal arterial resistor, arterial compliance, peripheral resistor, and venous compliance. The peripheral resistors consisted of a porous plastic sheet over which a motor-driven plate slid for the adjustment of cross sectional area of flow, and thus the resistance value. Each compliance unit incorporated a coil

spring-loaded piston moving inside a cylinder sealed with an elastomeric diaphragm. The model of the left ventricle consisted of a flexible, polymer sac inside a pressurization chamber. An artificial left atrium made of a flexible polymer sphere was connected upstream of the ventricle inflow valve. Introducer catheters were incorporated into both the ventricle and the atrium for the placement of catheter-tip pressure sensors. The caudal peripheral venous pool (PVP) consisted of a flaccid, polymer, 1 liter volume circuit bag taken from an anesthesia ventilator circuit, mounted inside a rigid cylindrical chamber. The non-linear response of the PVP was similar to that reported for natural veins.

The experiment was monitored with four flow probes, ten pressure transducers, a pair of ultrasonic crystals, six displacement transducers, and one accelerometer. Data from the September 1996 flight was analyzed during the first part of the 1996-1997 fiscal year. Regional circulating fluid shifts were measured during level flight (1-G), and throughout 0-G and 1.8-G periods. Regional flows and aortic outflow pressure were adjusted to physiologic values during 1g flight, and system response was observed during parabolic flight with no further adjustment or control. In the supine posture, the hydrostatic effect on the circulation is minimal and volume shifts were also small. In the launch posture, the PVP was elevated to 46 cm above the ventricle to simulate the elevation of the legs during a space shuttle launch. Fluid volume shifted away from the PVP to the regional MCS units between 0-G and 2-G. Regional volume shifts were most evident in the upright posture, with fluid shifting away from the cranial compliance chambers toward the caudal compliance chambers and the PVP with increasing acceleration. A leftward shift of the heart function curve was observed, with an increase in cardiac output in the presence of reduced filling pressure. This observation gives additional support to the hypothesis that this paradoxical increase in cardiac size and stroke volume, as reported from SLS-1 and SLS-2 missions, is caused by an increase in cardiac transmural pressure via a reduction in the intraplural pressure when the acceleration-dependent loading of the chest wall is removed.

While the above results were documented, an instability in the regional volumetric flow was discovered leading to the redesign of the regional MCS. The new design replaced the porous plastic and sliding plate with an open cell foam in a cylinder. The foam is compressed by a motor-driven piston and as the foam is compressed, the cell sizes are decreased and the resistance to fluid flow is increased. The compliance chamber design was not altered. This MCS was cheaper to build, easier to construct, and the resistors were substantially easier to replace. A prototype of the new design was flown on the KC-135A in April 1997 to prove that the new technology would work under all gravity conditions. After this flight, a new human cardiovascular model was built which also incorporated a fourth region, the Pulmonary MCS and an artificial right atrium and ventricle. The left ventricle was attached to the arterial vasculature and distributed to the Cranial, Central, and Caudal MCS units. The outflow of the regional arterial MCS units was connected to the artificial right atrium and flowed through the artificial right ventricle to the Pulmonary MCS. The outflow of the Pulmonary MCS was connected to the artificial left atrium to complete the cardiovascular system.

The PVP was also redesigned to make the volume shifting measurements more accurate. An ascending bellows from an anesthesia device was modified to allow volume measurements to be recorded using both pressure data and bellows displacement. The new PVP was mounted to allow easy movement from the supine and upright posture placement to the launch posture position. The PVP inflow was connected to the outflow of the Caudal MCS.

Ground-based 1-G data was collected on the newly designed hydraulic model in all three postures. The new system was monitored by five ultrasonic flow probes, seven catheter-tip pressure transducers, three port pressure transducers, nine displacement transducers, and one accelerometer. The volumetric flow instability was totally eliminated, and the new Pulmonary MCS and artificial right ventricle worked within human physiologic characteristics and values. The model was flown on four flights of the KC-135A for a total of 160 parabolas. Two KC-135A flights were on September 30, 1997 and the final two flights on October 1, 1997.

Hypotension and tachycardia are severe for many astronauts. Approximately half cannot tolerate a 10-minute stand test immediately after landing. Post-flight orthostatic intolerance first appeared after the fourth manned Mercury flight of only 34 hours and has occurred after flights of just nine hours. Most non-astronauts have

experienced orthostatic intolerance at one time or another and for some people the effects are chronic and debilitating. While long-term adaptations to microgravity may contribute to reduced tolerance, it is clear from the above results and from patients on Earth that short to intermediate-term effects must play an important role. Increased leg compliance, increased capillary permeability, deteriorated baroreceptor response, and hypovolemia are some of the causes that have been forwarded. The partial success of pre-landing ingestion of saline in preventing orthostatic intolerance indicates that hypovolemia is at least partially responsible; however, these results do not preclude the contributory effects of other factors. This project focuses on the effect of changes in hydrostatic pressure on the cardiovascular system, an effect that is present not only in launch and landing for astronauts, but also during changes in posture for people on Earth. Further study of this mechanism may lead to more effective countermeasures for all sufferers of orthostatic intolerance.

FY97 Publications, Presentations, and Other Accomplishments:

Sharp, M.K., Pantalos, G.M., Gillars, K.J., O'Leary, S., and Woodruff, S.J. "Modeling of cardiovascular response to weightlessness." BED-Vol. 35, 1997 Bioengineering Conference ASME, Sunriver, OR (June 11 - 15, 1997).

RRR-alpha-tocopheryl Succinate Modulation of TGF-beta and the TGF-beta Receptors on Human Myelocytic Leukemia (HL-60) Cells

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UPN/Project Identification: 199-99-27-02
Initial Funding Date: 1994
FY 1997 Funding: \$22,000

Solicitation: GSRP 95
Expiration: 1997

Task Description:

The overall goal of this task is to conduct basic research on the role of RRR-alpha-tocopheryl succinate (vitamin E succinate, VES), one derivative of the natural vitamin E (RRR-alpha-tocopheryl), on the biomodulation of the immunoregulatory transforming growth factor-beta (TGF-beta) system, using a human myeloid cell model in vitro. Specific aims are to investigate: (1) the regulatory effect of VES on TGF-beta isoform expression, and (2) the regulatory effect of VES on the expression of the cell surface TGF-beta receptors in human myelocytic leukemia (HL-60) cells.

Accomplishments made during the FY97 and previous years on the task were results showing that VES treatments affect TGF-beta production, activation, and receptor binding in HL-60 cells. HL-60 myeloid leukemia cells produce constitutive amounts of TGF-beta that can be activated from its latent form by VES, but not by a vehicle control. The induction of active TGF-beta can be seen in a dose and time dependent manner. One specific protein activating TGF-beta was also seen to increase after VES treatment. However, the concurrent mRNA data showed no significant change in TGF-beta mRNA levels. VES also increases the binding of the TGF-beta receptors in HL-60 cells, while modestly increasing the amount of TGF-beta Receptor Type II protein. Results have shown a relation of TGF-beta to the programmed cell death process (apoptosis), which is a current, intriguing topic in basic biological research. Neutralizing TGF-beta with specific antibodies can reduce the apoptosis induced by VES. At the end of the task in June 1997, the specific goals were completed with some new questions raised. One question is to distinguish between the effects on differentiation and apoptosis in HL-60 cells after VES treatment. TGF-beta is known to be involved with both processes for various cell lines. Another question is how the TGF-beta system is involved in apoptosis or what the signaling mechanisms are for apoptosis through the TGF-beta system. The answers will provide insight to the step by step response mechanisms involved following VES treatment in HL-60 cells.

The goal of this research was to understand the immunomodulator TGF-beta using a human myeloid leukemia cell model. The control of TGF-beta by VES allows for a possible therapeutic for cancer diseases on Earth or an understanding of the immune system in the environment of space. This research can relate the strong effects on the immune system by space to those of the potent immunomodulator TGF-beta. Investigating the effects on TGF-beta by vitamin E succinate can provide insight to the mechanisms involved in its control. By understanding the effects on the TGF-beta system and programmed cell death (apoptotic) mechanisms by VES in hematopoietic cells, this research can add information to basic biological processes.

FY97 Publications, Presentations, and Other Accomplishments:

Heim, K.E., Herbert, B., Sanders, B.G., and Kline, K. "RRR-alpha-tocopheryl succinate modulation of PAI-1 and PAI-2, uPA, and the TGF-beta system in MDA-MB-435 human breast cancer cells and myeloid HL-60 cells." (Poster) The University of Texas M.D. Anderson Cancer Center 49th Annual Symposium on Fundamental Cancer Research entitled, "Regulatory Mechanisms in Growth and Differentiation" (October 22 - 25, 1996).

Herbert, B., Sanders, B.G., and Kline, K. "Combinations of RRR-alpha-tocopheryl succinate and retinoids modulate growth and apoptosis in HL-60 cells: A role for a possible RAR-independent mechanism." (Poster) The University of Texas M.D. Anderson Cancer Center 49th Annual Symposium on Fundamental Cancer Research entitled, "Regulatory Mechanisms in Growth and Differentiation" (October 22 - 25, 1996).

Osteoblast Integrins and Osteoblast Function in Low Gravity

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1995

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

Space flight exploration and associated experiments have shown that human bones experience losses in density during inertial unloading, primarily due to the demineralization. This research project attempts to examine the effect of reduced force on the vertebrate skeleton by studying osteoblast function and structure in low gravity and the role played by integrins in bone deposition. Cultured chicken calvarial osteoblasts are cultured under static 1-G in the laboratory, space flight, clino-rotation, and centrifugation. Electron microscopy of the cells and resulting matrix will identify changes in osteoblast function and structure. Immunocytochemical assays will investigate differences in protein concentrations, focusing on integrin expression.

Transmission or scanning microscopy revealed that the osteoblasts fixed 3 hours into flight exhibited differences in attachment patterns on coverslips and were more vacuolated than their control counterparts. Three hour flight cells displayed a swirled appearance of substrate attachment, whereas control cells were arranged in a more parallel manner. After 3 days in microgravity, cells were fewer in number, of an apparent different shape, and even more vacuolated than controls. These observations with a chick culture model would suggest that the extreme launch environment and/or low gravity rapidly and adversely affect osteoblasts in terms of their phenotype, growth, and development. Results may also indicate that forces encountered by humans at launch and in microgravity may exert similar effects on bone.

In addition to gaining a better understanding of the bone loss that astronauts experience as a result of space flight, this research may lend insight into several terrestrial skeletal diseases, including osteoporosis, osteopenia, osteopetrosis, hypercalcemia, hypercalcuria, and Paget's disease. Moreover, this research will help identify the role of gravity (hyper-, hypo-, and normal 1-G) in the development, growth and differentiation in osteoblasts.

FY97 Publications, Presentations, and Other Accomplishments:

Kacena, M.A., Hodgens, K.J., Block, D.J., Todd, P., and Landis, W.J. "Studies of osteoblasts cultured aboard Shuttle Mission STS-77." (Abstract) ASGSB Bull. (1997).

Vestibular Influences on Sympathetic Outflows to Different Organs and Vascular Beds

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UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1997

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

Post-flight orthostatic hypotension poses a serious impediment to space exploration and maybe a result of plasticity in vestibular-autonomic interactions. This project will examine vestibular influences on sympathetic outflows that may play a role in correction for orthostatic hypotension. The first project will involve electrophysiological recordings from sympathetic nerves innervating vascular beds throughout the body during labyrinthine stimulation. The second project will involve blood catecholamine measurements in response to vestibular stimulation.

Though the work on this project is in its early stages, some interesting data have already been gathered. The most intriguing finding is that the different sympathetic nerves are differentially influenced by the vestibular system. For example, nerves that innervate the kidney are affected to a greater degree than the nerves innervating the head and neck vasculature. This result raises the possibility that during upright postural changes, the blood is shunted away from certain visceral beds (e.g., the kidney) to provide adequate perfusion of the brain. Redistribution of blood flow in such a manner may prevent the development of orthostatic hypotension under normal conditions. This finding raises several important questions including: 1) What are the central nervous system sites that mediate the observed changes in sympathetic nerve activity in response to vestibular stimulation? 2) Do the changes in the sympathetic nerve activity actually cause differential blood flow changes to various organs? 3) Can abnormal blood pooling within the visceral vascular beds lead to the onset of orthostatic hypotension during upright postural changes?

The goal of this research is to gain greater insight into the mechanisms that may lead to the development of post-flight orthostatic hypotension, a major health problem associated with the return from space. The aim of this project is to investigate the basic mechanisms of vestibulo-sympathetic interactions under normal conditions. Such knowledge is essential to the development of new and effective treatments for post-flight orthostatic hypotension.

FY97 Publications, Presentations, and Other Accomplishments:

Bolton, P.S., Kerman, I.A., Woodring, S.F., and Yates, B.J. "Influences of neck afferents on sympathetic and respiratory nerve activity." (Abstract) Soc. Neurosci. Abstr., 23, 753 (1997).

Kerman, I.A. and Yates, B.J. "Adrenal nerve responses to electrical vestibular stimulation." (Abstract) Soc. Neurosci. Abstr., 22, 92 (1996).

Kerman, I.A. and Yates, B.J. "Regional and functional differences in sympathetic nerve responses to electrical vestibular stimulation." National MD/PhD Student Conference, Aspen, CO (1997).

Kerman, I.A., Spiropoulos, J., and Yates, B.J. "Regional and functional differences in sympathetic nerve responses to electrical vestibular stimulation." (Abstract) Soc. Neurosci. Abstr., 23, 1518 (1997).

Yates, B.J., Steinbacher, B.C., Kerman, I.A., Jian, B.J., Woodring, S.F., and Miller, A.D. "Vestibular influences on autonomic control: Role in maintenance of homeostasis." International Workshop on Motion Sickness, Marabella, Spain (1997).

Immunotoxicity of Hydrazine

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UPN/Project Identification: 199-99-27-11

Solicitation: GSRP 96

Initial Funding Date: 1996

Expiration: 1999

FY 1997 Funding: \$22,000

Task Description:

Hydrazine (H_2N-NH_2) decomposes exothermically in a controlled manner in the presence of a catalyst, thus emitting gases for thrust or pneumatic power. NASA employs hydrazine to propel space shuttles and satellites and anticipates using it aboard the International Space Station. However, this reactive compound poses potential health risks both to ground crews that fuel the shuttles or satellites and to astronauts, who may inadvertently introduce hydrazine vapor into the cabin of a spacecraft following extravehicular activities. Hydrazine exposure has been implicated in immunomodulation, upregulation of some immune functions and downregulation of others. Furthermore, accidental dermal exposure to this compound can cause contact dermatitis, a hypersensitive reaction, and a systemic erythematosus lupus-like syndrome, an autoimmune reaction, in susceptible individuals. However, no study has addressed the immunological effects in humans or animals following inhalation of hydrazine. The purpose of this investigation is to elucidate potential mechanisms by which inhaled hydrazine affects the immune system both locally and systemically.

Our working hypotheses are: (1) inhalation of hydrazine causes both local and systemic effects on the immune system of an exposed individual, and (2) hydrazine can induce a systemic lupus erythematosus (SLE)-like syndrome via a Th2-dependent pathway, and inhalation exposure to hydrazine poses a significant risk to susceptible individuals. Experiments include *in vitro* studies of cultured human blood monocytes and alveolar macrophages and *in vivo* studies using murine models.

One of our aims is to characterize the effects of hydrazine on alveolar macrophages that function in the innate immune response as phagocytic cells in the lung. These cells also secrete cytokines, such as interleukin-1 (IL-1), which cause a wide variety of effects on differentiation and function of cells involved in both inflammatory and immune responses. Our second aim is to determine whether inhalation of hydrazine induces an SLE-like disease in susceptible individuals via a Th2 pathway.

There are two subsets of T-helper (Th) cells, Th1 and Th2; Th1 involves cytotoxic T-lymphocytes (cellular immunity), while Th2 involves T-cell activation of B-cells to produce antibody (humoral immunity). SLE is an autoimmune condition in which antigen, DNA, and its associated proteins, is constantly available. Antibodies that are produced to these specific common cellular components form complexes. These immune complexes are deposited in blood vessels in the glomerulus of the kidney and in other organs as well as in the joints. This deposition and the body's response to it cause tissue damage. Thus, SLE involves humoral immunity (Th2-mediated).

Preliminary studies have been examining the direct effect of hydrazine hydrate and hydrazine sulfate on human buffy-coat cells (macrophages and lymphocytes). We are investigating expression of cytokines in these cells treated with various doses of hydrazine after 6, 24, and 48 hours by enzyme-linked immunoassays (ELISAs) specific for these interleukins: IL-1, IL-4, and IL-12. Expression of IL-4 favors the Th2 pathway, while expression of IL-12 favors the Th1 pathway. Since SLE is mediated by the Th2 pathway, we expect to see an increase in IL-4 in the hydrazine-treated cells. We will determine the ratios of Th1 to Th2 cells by flow cytometry in mice treated with hydrazine by intratracheal instillation. Furthermore, we will use ELISAs to detect antibodies to DNA and assess for antinuclear antibodies in cells from these animals.

To study the immediate effects of hydrazine on immune cells in the lung, we have exposed mice to hydrazine intratracheally. Alveolar macrophages are obtained by lung lavage four hours after exposure to various doses of hydrazine. Preliminary results indicate that exposure causes apoptosis, programmed cell death, of some macrophages and mitosis of others. There are two subtypes of alveolar macrophages, suppressor and activation macrophages. If suppressor macrophages are dying and activated macrophages are dividing, upregulation of the immune response is predicted. We plan to use flow cytometry to determine alterations in the ratios of the two subsets in response to hydrazine exposure.

In cells from animals treated with higher doses of hydrazine, there is also some necrosis. When cells die by this means, cellular contents are released, including DNA and its associated proteins, thus exposing these constituents to cells involved in the immune response. This phenomenon is more pronounced in BALB/c mice than in C57/black mice, indicating the potential role for genetic predisposition in development of an immune response to inhaled hydrazine. We are comparing the effect of inhalation exposure to hydrazine in three strains of mice. The A strain mice should be most susceptible to a systemic lupus-like disorder, C57/black mice should be resistant, and the BALB/c mice should be intermediate between the other two strains.

Very dilute solutions of hydrazine react directly with deoxyribonucleosides *in vitro* under physiological conditions of temperature and pH. The reaction is catalyzed by iron and iron-containing biomacromolecules; catalase is the most active iron-containing compound in causing degradation of the bases [Lam, C-W., J. Latch, and J. James, March 1996, *In vitro* Reactions of Hydrazine with Deoxyribonucleosides, *The Toxicologist*, 30 (1), 237, (Abstract No. 1251)]. Hydrazine is known to form hydrazyl radicals in a variety of systems containing metal ions, and free radicals have been demonstrated to damage DNA, lipid, and protein. Thus, if hydrazine forms free radicals *in vivo*, a variety of types of damage are possible. Since autoantibodies to DNA and antinuclear antibodies are associated with SLE, it is possible that the interaction of hydrazine with DNA and/or DNA-associated proteins is involved in the SLE-like disease that can follow hydrazine exposure. We plan to use the triple-quad or two-dimensional high performance liquid chromatography to further analyze these DNA adducts in an *in vivo* system.

Hydrazine inhalation poses potential health risks both to ground crews that fuel shuttles or satellites and to astronauts, who may inadvertently introduce hydrazine vapor into the cabin of a spacecraft following extravehicular activities. It has been proposed that hydrazine vapor could condense on spacesuits and be carried into the craft resulting in exposure levels of approximately 50 parts per million (ppm). The spacecraft allowable concentration (SMAC) for hydrazine for a 180-day exposure is only 0.004 ppm. Therefore, the potential health effects of inhaled hydrazine are of critical interest to NASA. When it is combined with water, hydrazine serves as a source of gas to drive a turbine in the F16 aircraft's emergency power unit. Therefore, potential adverse effects of this compound are also of importance to the armed forces.

Hydrazine has many other applications. It is used in explosives and is a powerful reducing agent. Hydrazine derivatives—salts and hydrazones—are used in both growth retardants and growth enhancers for plants; they are also used as insecticides, fungicides, herbicides, bactericides, surfactants, detergents, plasticizers, and pharmaceuticals. Pharmaceutical applications include tuberculosis treatment, high blood pressure alleviation, and cancer therapy. Hydrazine is naturally produced by some bacteria, algae, and fungi and is found in side-stream tobacco smoke as a result of bacteria and fungi present in the curing process. In addition, hydrazine is a metabolite of some toxic

mushrooms. Therefore, the general public can be exposed to hydrazine and experience potential adverse health effects.

FY97 Publications, Presentations, and Other Accomplishments:

Latch, J. "Immunotoxicity of hydrazine." Oral presentation, Johnson Space Center (October, 1996).

Minimum Surface Effect Microactuator for Dexterous Micromanipulation

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UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1996

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

This project entails the development of a tendon-based microactuator suitable for use in telerobotic systems allowing dexterous interaction with microscopic environments. The actuator design incorporates a piezoelectric ceramic (PZT) stack power transducer coupled to a three-stage, nonlinear, deformation-based transmission that converts the PZT output to a nearly constant one Newton force through a stroke of one millimeter, in an open-loop stable manner. The package incorporates separate strain-gage based force and position sensors and is designed to be controllable to one micron positioning resolution or one milliNewton of force.

Analysis leading up to the design of the actuator led to the knowledge that the physics of scaling cause undesirable surface forces such as friction and backlash to become increasingly dominant as the size of an object decreases. Since these effects are witnessed in all sliding-contact interfaces (such as those found in conventional hinges), the current work focuses on creating a mechanism that is devoid of any sliding contact surfaces, but rather relies on selectively compliant locations to create deformation-based revolute joints. In doing so, a complex structure is created whose members bend at desired locations to emulate a kinematic linkage. The current research has indicated that PZT stack actuators serve as good power transducers for this type of application because of their large power density, open-loop stable behavior, and strain-based output that does not exhibit stick-slip or backlash behavior observed in other actuators. However, PZT actuators do have undesirably nonlinear (although smooth) characteristics that must be compensated for with a similarly nonlinear transmission. Therefore, the design of the actuator yielded a multi-stage structure that imitates the behavior of a complex kinematic linkage to cancel the nonlinear effects of the power transducer.

During FY97, finite element analysis and a numerical design optimization were completed for the actuator. The prototype was then fabricated, instrumented, and tested. Although the mechanism failed to meet its design criteria, the design of the actuator and analysis following testing have yielded helpful conclusions regarding the design of multi-stage kinematic structures, including: the necessity of proper impedance matching between stages of a deformation-based linkage, effective linkage configurations for creating nonlinear transmissions, and better insight into flexure design. The results of this work should provide valuable guidance in the future design of structure-based actuators and machines.

This research is part of a larger project to design a teleoperated micromanipulator that will allow a researcher to dexterously interact with an environment being viewed through a stereo microscope. The researcher's position inputs to the micromanipulator will be input through a conventional-scale manipulator, or haptic interface, and

scaled down to the micromanipulator. Forces experienced by the micromanipulator will conversely be amplified and reflected back through the haptic interface to be felt by the user. Thus, the illusion will be created for the researcher that physical interactions with very small objects are taking place on a conventional scale. Such technology might be considered the mechanical analog of the microscope, allowing deft interaction with micro-scale environments. This work could potentially benefit a wide range of researchers in the field of life sciences, including work in biology, the production of crystals in microgravity, and eye surgery and neurosurgery. Because the interface and micromanipulator are not required to be in spatial proximity, a scientist on Earth using this technology would be able to interact with a microscopic experiment taking place in space.

FY97 Publications, Presentations, and Other Accomplishments:

Goldfarb, M. and Lipsey, J. " Design of a minimum surface-effect tendon-based microactuator for micromanipulation." IEEE Conference on Robotics and Automation (1997).

Microvascular Alterations in Simulated Microgravity

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1997

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

We hypothesize that alterations in vascular smooth muscle function and/or structure are responsible for the orthostatic intolerance experienced by astronauts post-flight. The proposed experiments will examine *in vitro* vascular responses (diameter changes) to vasoconstrictors, transmural pressure, and shear stress in resistance arteries and small veins from hindlimb-suspended and control rats (hindlimb suspension is an accepted method of microgravity simulation). We hypothesize that isolated vessels will exhibit attenuated constriction to all vasoconstrictors, increased distensibility to transmural pressure, and enhanced vasodilation to shear stress. To determine the mechanism of attenuated vasoconstriction, we will examine vascula and smooth muscle cell morphology, actin-myosin contents, and contractile apparatus activation (myosin light chain phosphorylation).

We are currently performing 4-week hindlimb-suspension on the first group of rats. After this period, vessels will be isolated from the mesentery and hindlimb to measure functional responses. If there is an attenuated contractile response to all vasoconstrictors (norepinephrine, angiotensin II, KCl) as hypothesized, we will examine the smooth muscle morphology, contractile components, and the ability of the vasoconstrictor signaling pathway to phosphorylate myosin light chain (the essential molecular event for smooth muscle cell contraction). If vasoconstriction is only attenuated to receptor-dependent vasoconstrictors (norepinephrine, angiotensin II), we will examine adrenergic receptor number and affinity, G protein content, and G protein-receptor coupling.

Our studies are aimed at understanding the vascular molecular mechanisms of orthostatic intolerance after space flight. Results from this study are not only applicable to microgravity-induced orthostatic intolerance, but also orthostatic intolerance after periods of bed rest.

Musculoskeletal Countermeasures to Spaceflight

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Solicitation: GSRP 96

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Expiration: 1999

FY 1997 Funding: \$22,000

Task Description:

There currently exists no practical and reliable technique for prevention or reversal of osteoporosis (bone loss) that occurs due to prolonged exposure to less than Earth's gravity. Although countermeasures to space deconditioning have been developed (ergometer, treadmill, APenguin@ suit, and lower body negative pressure), these measures do not completely solve the bone loss problem. Bone mass accounts for 75-85% of the variability found in bone strength, and the ultimate medical consequence of a reduction in bone mass is an increased risk of fracture. Without practical and effective measures to counter the debilitating effects of bone loss, astronauts may not be able to function normally upon return to Earth's gravity after extended survival in non-terrestrial (space, Moon, Mars) environments. The proposed research will attempt to increase the knowledge of preserving skeletal function by introducing a novel therapy (optimal impulse waveform). Additional research may also be performed examining the effect of resistive exercise on bone and muscle.

The means necessary to preserve skeletal function during long-term exposure to microgravity and low-gravity environments are still poorly understood. Recent research has focused on the precise and quantitative characterization of the mechanical behavior of cancellous and cortical bone, and the adaptive changes which occur during space flight, aging, menopause, chronic inactivity, and exercise. Presumably such adaptations occur in response to changes in the bone's mechanical stimulation. New strategies, which involve the use of discrete sine waves to form a mechanical stimulus signal, suggest that the frequency content of the mechanical stimulus is very important in terms of the skeletal remodeling response, but such approaches do not necessarily provide optimal mechanical excitation of the skeleton's remodeling response. To circumvent these inadequacies, a mechanical stimulation system based upon an optimal impulse waveform (OIW) is proposed. The concept of the OIW is to create a computer-driven impulse waveform containing energy tuned to specific frequencies. The phases at each frequency are optimized so that the resulting waveform delivers the desired mechanical force to the skeleton. These mechanical forces will be applied to the heel bones of the rabbits and possibly humans. The amplitude of the load delivered will be very small (less than one body weight), but will achieve maximal effectiveness since the frequency content of the impulse signal will be tuned to the natural frequency of the skeleton. Additional research may focus on resistive exercises performed by normal subjects using a 3-axis dynamometer and/or a Cybex lift station to stimulate the trunk and a reciprocating "stepper" cycle to stimulate the lower extremities. By establishing clinically useful methods for prevention of osteoporosis, this study will provide practical countermeasures for bone loss associated with not only a reduced gravity environment, but with aging and disuse as well.

The first task was to perform a review of the literature on bone mechanical stimulation in order to determine the latest developments in the field. Following the literature search, an experimental protocol was developed based on the OIW concept for animal experiments which has been approved by the University's IRB. Initial experiments will examine the effect of mechanical impulses on the immobilized hind limbs of mature rabbits. One of the hind limbs of each rabbit will be immobilized, but only half the animals will receive the OIW protocol. Densitometric, mechanical, and morphologic differences between the immobilized limbs, stimulated immobilized limbs, and non-immobilized limbs will be evaluated using a paired observations t-test. Before testing of live rabbits could begin, the strain frequency response of the hind limb bones needed to be determined. An experiment has been completed that examined the transmissibility of loads engendered at different frequencies to rabbit tibias using a pneumatic impulse delivery device. Preliminary results indicated that the natural frequency (highest transmissibility) of the rabbit tibia is in the 15-30 Hz range. This range has been observed by other authors for different species. We hypothesize that excitation in this range will cause a significant enhancement in bone mass in comparison to other frequency ranges. Additional *in vitro* and *in vivo* experiments will be conducted on immobilized rabbit hind limbs using an electronic mechanical impulse delivery device. The live animal experiments which examine the effect of the OIW bone stimulation protocol will be performed in conjunction with another study, which focuses on the effects of immobilization on the Achilles tendon mechanical properties.

Besides work being accomplished at the University, this summer (5/17/97 – 9/5/97) was spent working in the Exercise Physiology Laboratory at NASA-Johnson Space Center (JSC) located in Houston, Texas. The project undertaken at JSC focused on the Austrian isokinetic exercise device, MOTOMIR. The MOTOMIR experiment is designed to examine the concept that intensive resistive exercise will be able to maintain skeletal muscle strength and prevent or retard skeletal muscle atrophy during space flight as well as evaluate the effectiveness of resistance exercise on net bone change. MOTOMIR provides a resistive exercise workout while allowing the investigator to record physiological and neuromuscular parameters not only before and after flight, but during microgravity in a valid, standardized, and reproducible manner. This device is currently stored on the Mir space station and investigators are interested in having both cosmonauts and astronauts use the device during the joint stays aboard the station. The project included obtaining the MOTOMIR from the Austrians, setting up the device in the Exercise Physiology Laboratory, defining the exercise protocol, and producing the appropriate paperwork to allow the investigation to occur on Mir. The MOTOMIR protocol established isolates the quadriceps muscle in a single leg so that the effectiveness of the resistive exercise can be determined by comparison with the non-exercised muscle. If this resistive exercise protocol works on the muscles of one limb, the results may be extrapolated to other skeletal muscle groups. This data will then provide a blueprint by which the protocol is effective or needs to be perturbed before moving to the implementation of a whole body resistive exercise countermeasure for subsequent Mir and International Space Station flights. In addition to the MOTOMIR project, a variety of exercise devices both currently used and planned to be used were observed. One of these resistive exercise devices may be evaluated as part of this project by examining muscle and bone changes and comparing with other exercise devices.

Clinically, osteoporosis is a disorder which may be defined as a decrease in bone quantity associated with an increased risk for fracture; a close association between bone mineral loss due to osteoporosis and the risk of fracture has long been established. Osteoporosis is a well recognized public health problem of increasing proportions, affecting both the appendicular and axial skeleton of adults. Skeletal structures which are comprised primarily of trabecular bone (vertebral bodies, long bone metaphyses and epiphyses) appear to be particularly at risk, and can exhibit as much as a 2% per week loss in bone mass during chronic immobilization and bedrest. Over 1.2 million fractures occur in the United States each year, including over 500,000 cases of vertebral fracture, and 200,000 cases of hip fractures, one-third to one-half of which occur in women over the age of 65. Thirty percent of subjects reaching the age of 80 years will have hip fractures, 16% of patients suffering hip fractures will die within six months of having the fracture and up to 50% of patients with a hip fracture will require long-term nursing care. The personal and medical costs associated with osteoporotic fractures is expected to increase dramatically in the next two decades, since the number of individuals over the age of 65 has been predicted to double by the year 2010. By establishing clinically useful methods for prevention of osteoporosis, this study will provide practical countermeasures for bone loss associated with not only a reduced gravity environment, but with aging and disuse as well.

Sleep Restriction and Mechanisms of Orthostatic Tolerance

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UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1997

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

Post-flight orthostatic intolerance is characterized by exaggerated tachycardia, exaggerated stroke volume decreases, and attenuated vasoconstriction. We have found augmented sympathetic activation and attenuated vasoconstrictor responses to orthostasis following sleep restriction. Since astronauts often experience significant sleep restriction and interruption, we hypothesize that this sleep restriction can lead to augmented sympathetic neural activation and attenuated vasoconstrictor responses to orthostasis, and thereby contribute to orthostatic intolerance. To test this hypothesis, we propose to study 24 human subjects before and after 4 days of sleep restriction (4 hours total sleep time per night) and determine the effect of sleep restriction on the sympathetic neural and vasoconstrictor responses to baroreflex stimulation and lower body negative pressure and its effect on orthostatic tolerance. We anticipate that sleep restriction will result in exaggerated sympathetic and attenuated vasoconstrictor responses to orthostasis and reduced orthostatic tolerance.

Preliminary studies have been completed on 8 of 24 subjects, evaluating the effects of 4 days of sleep restriction (4 hours per night) on autonomic control of the cardiovascular system. These preliminary studies suggest that: 1) baseline sympathetic nerve activity, vascular resistance and blood pressure are not altered by 4 days sleep restriction, and 2) orthostatic tolerance may be impaired in selected subjects, but is not consistently affected by sleep restriction.

This project has two parts of potential significance for Earth benefits. First, we are addressing the possible mechanisms of vasovagal syncope. One underlying hypothesis of this project is directed specifically at this question. That is, exaggerated sympathetic neural activation can increase susceptibility to syncope. Both basic science and clinical data are consistent with this hypothesis and if the results support the hypothesis, it may help guide the therapy of individuals at risk for neurally-mediated syncope (both post-space flight and on Earth). Second, the effects of sleep deprivation/restriction are of great potential interest, since many individuals in the working world are often faced with periods of sleep restriction.

Muscle Oxygenation as an Objective Method to Evaluate and Optimize the Space Station Glovebox Design

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UPN/Project Identification: 199-99-27-09

Solicitation: GSRP 96

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FY 1997 Funding: \$22,000

Task Description:

Localized fatigue of the hand, arm, shoulder, and neck are caused by sustained static loading of a specific muscle, tendon, and joint. Such sustained exertions over time, can increase muscle pressure, decrease local blood flow and oxygenation, and may cause pain, discomfort, and functional deficits of the involved limb. Constrained working postures pose high risks for upper extremity fatigue and discomfort. People who work in Gloveboxes are subject to postural constraints. Similarly, astronauts who work in a glovebox are subject to postural constraints while performing tedious and repetitive tasks. For example, an astronaut suffered from severe shoulder pain in microgravity which was diagnosed as long thoracic nerve palsy. Postflight medical analysis of the astronaut led to the conclusion that static shoulder abduction and internal rotation while working in the Glovebox placed the thoracic nerve under tension, possibly causing deoxygenation and dysfunction of this nerve. Analysis of the Spacelab Glovebox determined that narrow gloveports and constricting arm cuffs as well as prolonged use of the Glovebox were primarily responsible for the astronaut's nerve impingement syndrome. A Life Sciences Glovebox is currently being designed at the Ames Research Center for use on the space station. Thus far, Glovebox design concepts have been only evaluated subjectively through operational testing and computer modeling. No Glovebox for use during space flight has been evaluated objectively with physiologic measures. The purpose of this research investigation is to use oxygenation as an objective physiologic parameter to study forearm and shoulder muscle fatigue during work in the Glovebox for an extended period of time, and to apply this measurement of oxygenation to optimize Glovebox design.

To study forearm and shoulder muscle fatigue during glovebox work using near infrared spectroscopy (NIRS), we modified the sensor so that it can be used to study small and superficial muscles such as the extensor carpi radialis muscle of the forearm. Also, because Glovebox work involves low levels of muscle contraction (less than 20% maximum voluntary contraction, MVC), we needed to determine whether NIRS is sensitive to changes in muscle oxygenation during low levels of contraction. To address these issues, we conducted a study and hypothesized that muscle oxygenation (TO_2) decreases significantly at low levels of forearm static contraction. In nine healthy male and female subjects, we measured altered TO_2 noninvasively using NIRS. The NIRS probe was placed over the forearm extensor muscle and gently secured with an ace wrap. After one minute of relaxed, baseline measurements, four different loads (randomly ordered) were placed just proximal to the metacarpophalangeal joint such that subjects isometrically contracted the extensor muscle at 5%, 10%, 15%, and 50% of MVC for 1 minute each. A three minute recovery period followed each contraction level. At the end of the protocol, with the NIRS probe still in place, an ischemic TO_2 was obtained to establish a TO_2 zero

level for each subject. NIRS data were normalized to a relative scale between the physiologic minimum (0%) established during ischemia and at baseline (100%). Mean TO₂ decreased from resting baseline (100% TO₂) to 89 ± 4% (SE), 81 ± 8%, 78 ± 8%, and 47 ± 8% at 5%, 10%, 15%, and 50% MVC, respectively. TO₂ levels at 10%, 15%, and 50% MVC were significantly lower ($p < 0.05$) than baseline values. This study demonstrates a significant reduction in TO₂ even at sustained contraction levels as low as 10% MVC. Tissue deoxygenation during prolonged isometric muscle contraction may play an important role in the development of work-related muscle fatigue and pain. Static or dynamic contraction with inadequate recovery time may sustain elevated intramuscular pressures, and reduce blood flow and TO₂, and cause muscle fatigue and pain.

This study indicates that muscle oxygenation decreases significantly during low levels of muscle contraction and if astronauts working in the Glovebox also are subjected to similar low levels of contraction for prolonged periods, then it is highly likely that muscle fatigue and dysfunction will result. Furthermore, the cephalad fluid shifts and low blood pressure in the hands imposed by the microgravity environment may predispose astronauts to further muscle fatigue, pain, and dysfunction. Therefore, studies to determine deoxygenation as a potential cause of muscle fatigue are critical.

From our study we know that oxygenation is reduced during low levels of muscle contraction, that NIRS is sensitive to low levels of oxygenation, and that muscle fatigue as determined subjectively occurs during low levels of muscle contraction. However, new questions which warrant investigation are: (1) How do we measure fatigue objectively? (2) Is muscle fatigue directly a result of reduced muscle oxygenation?

Muscle fatigue is a work-related problem among workers in space and on Earth. On Earth, Gloveboxes are used widely in high technology settings: information technology, pharmaceutical, and biotech industry, government research laboratories, hospitals, as well as chemical and nuclear plants. It is estimated that there are over 250,000 Gloveboxes in the U.S. Most of the off-the-shelf Gloveboxes are inadequate to meet general needs and therefore, Gloveboxes are custom-designed to accommodate specific tasks. Although Glovebox workers on Earth stand or sit to ease work-induced fatigue, it is difficult to maintain neutral neck, shoulder, and upper extremity posture to produce quality work over extended periods of time. Therefore, using a physiologic measure such as muscle oxygenation to aid in the design of Gloveboxes will benefit Glovebox workers on Earth as well in space. Furthermore, if we can measure oxygenation noninvasively, it provides a valuable tool to assess muscle fatigue and discomfort.

FY97 Publications, Presentations, and Other Accomplishments:

Murthy, G., Kahan, N.J., Hargens, A.R., and Rempel, D.M. "Forearm muscle oxygenation decreases with low levels of voluntary contraction" in "Environmental Ergonomics: Recent Progress and New Frontiers." Edited by: Shapiro, Y., Moran, D.S., and Epstein, Y. Freund Publishing House, Ltd., London, England, pp 35-38 (1996).

Murthy, G., Kahan, N.J., Hargens, A.R., and Rempel, D.M. "Forearm muscle oxygenation decreases with low levels of voluntary contraction." *J. Orthop. Res.*, 15, 507-511 (1997).

Murthy, G., Kahan, N.J., Hargens, A.R., and Rempel, D.M. "Muscle oxygenation decreases with low levels of voluntary contraction." VII International Conference on Environmental Ergonomics, Jerusalem, Israel (October 26, 1996).

Murthy, G., Kahan, N.J., Hargens, A.R., and Rempel, D.M. "Forearm muscle oxygenation decreases with low levels of voluntary contraction." *Transactions of the 43rd Annual Meeting of the Orthopaedic Research Society*, Vol. 22(2): 702 (1997).

An Investigation of Composting Plant and Human Wastes in a Controlled Ecological Life Support System (CELSS)

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1997

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

This study will investigate the composting of plant and human wastes in a controlled ecological life support system (CELSS). Penn State Harrisburg will develop, operate, and analyze the dynamics of an in-vessel composting system, using a generated waste that will determine which operational settings (i.e., mixing and aeration) provide the most effective levels of biodegradation and the control of temperature, moisture, and the transfer of oxygen.

The NASA project, which began August 1, 1997, is still in the development stage of the bench-scale system. Experimental runs of this project will begin in December and end in the summer of 1998.

Not only will this project and the successful integration of a CELSS have potential benefits for the space program, but it may also provide answers to the science community as to how the Earth and its ecosystems work together to sustain life.

Estrogen Receptor Interactions in Uterine Cell Proliferation

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Funding:

UPN/Project Identification: 199-99-27-03

Solicitation: GSRP 95

Initial Funding Date: 1994

Expiration: 1997

FY 1997 Funding: \$22,000

Task Description:

In the uterus, estrogen causes a cascade of cellular events that result in synthesis of DNA, mitosis, and proliferation of uterine cells. To fully understand the mechanisms that cause these dramatic changes, we are focusing on the primary target of estrogen, the estrogen receptor (ER) protein and its interaction with other regulatory proteins. A recently developed system, the yeast two-hybrid system, is being used to investigate 1) whether the estrogen receptor protein can form a dimer (associate with another estrogen receptor), 2) identify the parts (domains) of ER responsible for dimerization, and 3) identify novel uterine specific proteins that interact with ER to affect its activity.

Using the yeast two-hybrid system, we have shown that the dimerization of the estrogen receptor is dependent upon the presence of estrogens or antiestrogens. The results of these experiments were published in October 1995 in *The Journal of Biological Chemistry*.

The domains responsible for ligand induced dimerization of ER have been localized. It appears that regions at the carboxy-terminal end of the molecule may inherently contain repressing sequences for interactions with ER and other regulatory molecules. These results are currently being confirmed with other methods. Several potential positives have been identified that interact with ER in screening experiments with uterine libraries. If these proteins are found to be true positives, the roles of these proteins in both the normal and neoplastic uterus will need to be determined.

Endocrine factors are thought to play key roles in physiological mechanisms of metabolic changes observed in astronauts during space flight and readaption to Earth's gravity. However, little is known about the primary targets of steroid hormones, particularly female steroid hormone receptors, and the regulatory mechanisms involved in their action. To fully understand the mechanism that causes these changes *in vivo*, the yeast two hybrid system was used to study the primary target of estrogen, the estrogen receptor. This research contributes to our basic understanding of molecular events in activation of the human estrogen receptor, and should be of particular interest towards the women's space program.

FY97 Publications, Presentations, and Other Accomplishments:

Peters, G.A. and Khan, S.A. "Estrogen receptor hormone binding domain interactions in yeast." ENDO '97, Endocrinology Research Conference, Minneapolis, MN (1997).

AOTF Spectrometer System for the Remote Detection of Plant Canopy Nutrient Stresses in a NASA CELSS

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1996

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

The acousto-optic tunable filter (AOTF) spectrometer at Penn State's Applied Research Laboratory (ARL) will be a useful tool for rapid scanning and remote analysis of plant canopies. Passive and active remote sensing spectra is to be correlated with specific nutrient stresses manifest within *in situ* leaf material. Reflectance spectra will be used to monitor the concentration of primary plant pigment and provide stress related information. Laser induced fluorescence (LIF) data will be used to monitor specific changes in plant physiology, metabolism, photosynthetic activity, and photopigment concentration. Optimization of spectral resolution, wave function normalization, and total imaging area will also be assessed in this study.

◆ Doctoral committee members selected: (1) Dr. Paul Walker, (advisor) Professor of Agricultural and Biological Engineering; (2) Dr. Russell Philbrick, (co-advisor) Professor of Electrical Engineering, Head of Remote Sensing Department; (3) Dr. Simon Gilroy, Assistant Professor of Biology; (4) Dr. Paul Heinemann, Associate Professor of Agricultural and Biological Engineering.

◆ Initial tests conducted with AOTF on standard light sources.

◆ Individual study created, Spectrometry Theory and Methods for Specific Applications. Topics included: atomic spectra, molecular spectra, scattering processes, basic electronics, and spectrometry instrumentation. Bulk of Ph.D. course work completed: Remote Sensing, System Simulation, Plant Physiology, Space Astronomy, Molecular Basis of Plant Development, Introduction to Electro-Optical Engineering, and Fundamentals of Remote Sensing

◆ Literature Review – have begun review of current journal articles and publications related to the remote sensing of plants and LIF of biological materials.

New questions – 1) How much radiant flux can be incident upon plant tissue without inducing damage? 2) Does a pulsed laser signal have significant advantage over comparable system with steady emission? 3) How to best account for differences in spectra resulting from the angle of inclination of plant material and for variation in plant parts?

This research will provide insight into the interaction of biological material with light and the use of remotely sensed data to predict plant vigor. Spectral signature correlation to plant stress and general system optimization will be helpful for the construction of a fully automated, remote sensing device to monitor the health and

development of vegetation. Such a device could be used to monitor crops, forests, and wildlife habitat here on Earth as well as plant-based bioregenerative life support systems used for the exploration of space.

Effects of Microgravity on Cell Mediated Immunity and Reactivation of Latent Viral Infections

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Funding:

UPN/Project Identification: 199-99-27-10

Solicitation: GSRP 96

Initial Funding Date: 1996

Expiration: 1999

FY 1997 Funding: \$22,000

Task Description:

The majority of humans are infected with herpesviruses (i.e., herpes simplex type-1 (HSV-1), cytomegalovirus (CMV), and Epstein-Barr virus (EBV)). These infections are characterized by an acute phase usually associated with minor morbidity and mortality followed by a chronic latent phase reflecting a balance between viral replication and the host immune response. Impaired cellular immunity has been associated with both mild and severe clinical symptoms due to reactivation or recurrence of these viral infections. Recent studies have demonstrated a decrease in cosmonauts' and astronauts' cellular immune function both during and after space flight. The hypothesis is that the combined effects of microgravity along with associated physical and psychological stress will reactivate latent EBV (B-lymphocytes) and CMV (urine) as well as decrease virus-specific T-lymphocyte immunity. The specific aims to test this hypothesis are: 1) collect blood, serum, and urine samples from astronauts before and after space flight; 2) measure specific antibody titers in serum samples; 3) determine the frequency and duration of latent virus shedding in pre-/post-flight samples using polymerase chain reaction (PCR) methodology and standard culture techniques; 4) determine T-lymphocyte immunocompetence using an EBV-specific autologous T-cell killing assay; and 5) characterize viral gene expression in EBV-infected B-lymphocytes. If increased viral reactivation is detected, then pharmacological measures can be developed and instituted prior to onset of overt clinical disease.

This project has received approval from the Johnson Space Center Institutional Review Board and has been manifested on space shuttle missions. Samples (peripheral blood and urine) have recently been collected from Shuttle crewmembers. Flow cytometric analysis of CD19 (B-lymphocytes) and CD3 (T-lymphocytes) showed little change at landing as compared to preflight values. White blood cells from peripheral blood samples were further separated into T-cell, B-cell, and monocyte subsets using magnetic beads and fixed for subsequent PCR analysis to determine the presence of herpesvirus DNA. Viral gene expression will be determined using reverse transcription (RT)-PCR. Specific antibody titers (EBV-viral capsid antigen, early antigen, and nuclear antigen) will be measured using indirect fluorescence assays. Viruses present in urine will be assayed using PCR and standard viral culture techniques. T-lymphocyte immunity will be assessed in future missions using an EBV-specific autologous T-cell killing assay. More subjects will be obtained in future shuttle missions comprised of 9- to 16-day flights.

The rapid and accurate diagnosis of herpesvirus infections (both primary and relapse) is extremely important. Infections such as EBV-associated lymphoproliferative diseases and herpes simplex encephalitis are severe and

without treatment fatal in many cases. This research project may provide new insights into the mechanisms of EBV reactivation and shedding. In addition, a novel method which rapidly quantitates the number of EBV-infected B-lymphocytes is being developed. Potential applications of this research project include development of rapid and sensitive diagnostic methods for identifying crewmembers at increased risk of illness.

FY97 Publications, Presentations, and Other Accomplishments:

Stowe, R.P., Mehta, S.K., Kaur I., Jones, M.L., Feeback, D.L., Barrett, A.D.T., and Pierson, D.L. "Effect of space flight on neutrophil function." 12th Man in Space Symposium, Washington, DC (1997).

The Role of Integrins in the Transduction of Gravitropism

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Funding:

UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1996

Expiration: 1998

FY 1997 Funding: \$21,965

Task Description:

Gravitropism can be divided into three events: perception, transduction, and response. Recent work on characean algae (Wayne et al., 1990, 1992) suggests that integrin-like molecules (a group of integral membrane proteins in the plasma membrane) may play a role in the transduction of a gravistimulus in plants. The purpose of this study is to examine the role of integrin-like proteins in the transduction of a gravitational stimulus in two important research models for gravitropism: *Chara*, an alga, and *Arabidopsis*, a flowering plant. Recent work in our laboratory has demonstrated that integrin-like proteins are present in these organisms. The specific aims of this proposed project are: 1) To examine the distribution and expression of integrin-like proteins in *Arabidopsis* and to study changes in expression that may result from reorientation in a gravitational field; 2) To determine if integrin-like proteins function in the transduction of gravitropism in *Arabidopsis*; 3) To examine the distribution and expression of integrin-like proteins in *Chara* rhizoids; and 4) To determine if integrin-like proteins function in the transduction of gravitropism in *Chara*. Successful completion of this study will provide insight into the signal transduction process in plant gravitropism and will determine if similar molecules are involved in transduction in both plant and animal systems. In addition, this ground-based study should contribute information relevant to recent space flight experiments of Dr. John Kiss on a Biorack project that were flown on STS-81 and STS-84.

Preliminary data obtained from immunofluorescence studies performed on *Arabidopsis* revealed that the integrin-like proteins are enriched in the root cap. Further immunofluorescence studies using a confocal microscope showed that the strongest signal emanates from plastids, both amyloplasts and chloroplasts. While immunoblotting demonstrates that integrin-like proteins are also present in plasma membrane fractions, fluorescent signal from the cell periphery is weak. Distribution of integrin-like proteins in space flight grown *Arabidopsis* seedlings does not appear to be significantly different from ground grown or RGDS tetrapeptide treated seedlings.

Distribution of integrin-like proteins in *Chara* has been documented previously by our lab. Integrin-like proteins are enriched at the rhizoid tip. Microinjection of *Chara* rhizoids with RGDS tetrapeptides results in complete inhibition of the gravitropic response. However, the inhibition appears to be a result of microinjection itself.

The gravitropic response of *Arabidopsis* seedlings is inhibited by the applications of an RGDS tetrapeptide sequence, but not by application of GGGG, SDGRG, RFDS, or RGES tetrapeptides. Inhibition is most evident

in vertically grown seedlings 2 hr after reorientation to a horizontal position. The inhibitory effect increases with increasing concentration.

Continued exploration of a structural basis for integrin-mediated signal transduction led to the discovery of an α -actinin-like protein and a talin-like protein that appear to colocalize with integrin-like proteins in *Arabidopsis* roots and shoots. Immunofluorescence and immunoblotting studies demonstrate that the α -actinin-like protein is recognized specifically by a polyclonal anti- α -actinin antibody and is enriched at the plastids and is present as a peripheral protein at the plasma membrane. Immunofluorescence studies also suggest that the talin-like protein colocalizes with the integrin-like proteins, but immunoblotting and immunogold staining are required for confirmation. Nevertheless, α -actinin may provide a direct linkage between integrin and actin filaments in animal systems. Therefore, an integrin/ α -actinin/actin link may provide competent structural support for signal transduction.

This study should provide insight into a basic biological plant function that is crucial to human survival on Earth and in long-term space flight: plant tissue formation. In animal systems, integrins play a key role in signal transduction in cellular functions such cell motility, cell surface adhesion, extracellular matrix formation, and morphogenesis. We seek information that should add to the understanding of how plants perceive and respond to gravity or to the lack of gravity, how proper root and shoot orientation is developed and maintained on Earth, and how microgravity will affect potential crop production in space. Basic knowledge of the biological processes leading to plant tissue formation may allow for maximization of tissue output with minimization of resources, a crucial issue for human survival on Earth and in living conditions in space.

FY97 Publications, Presentations, and Other Accomplishments:

Katembe, W.J., Swatzell, L.J., Makaroff, C.A., and Kiss, J.Z. "Immunolocalization of integrin-like proteins in *Arabidopsis* and *Chara*." *Physiologia Plantarum*, 99, 7-14 (1997).

Kiss, J.Z. "Gravitropism in the rhizoids of the alga *Chara*: A model system for microgravity research." *Biol. Bull.*, 192, 134-136 (1997).

Kiss, J.Z. "The potential role of integrin-like proteins in plant gravitropism." *Eur. Low Grav. Assoc., Abstract Book*, Paris, France, 30 (1997).

Swatzell, L.J., Weise, S.E., Makaroff, C.A., and Kiss, J.Z. "Co-localization of integrin-like and an actinin-like protein from *Arabidopsis*." *Plant Physiol.*, 114(S), 264 (1997).

Characterization of an Osteoblastic Scavenger Receptor

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UPN/Project Identification: not available

Solicitation: not available

Initial Funding Date: 1996

Expiration: 1998

FY 1997 Funding: \$22,000

Task Description:

It is well known that bone mass is progressively lost in humans and experimental animals under conditions of microgravity; remaining bone also appears to revert to a more immature phenotype. This bone loss is accompanied by hypercalciuria, which predisposes to renal calculi formation. It has been suggested that bone contains a "mechanostat" which senses changes in gravity and adjusts bone mass accordingly, altering the normal physiological equilibrium between bone formation and resorption. The osteoblast is the primary target for bone resorbing hormones (e.g., PTH, 1,25-(OH)₂ vitamin D₃, retinoic acid), and produces neutral proteases (notably collagenase-3) which degrades osteoid. We hypothesize that under conditions of reduced gravity, the regulation of collagenase-3 becomes aberrant. We have identified a specific collagenase-3 receptor on osteoblasts, fibroblasts, and chondrocytes from human and murine tissues. This receptor (which is a member of the low-density lipoprotein (LDL) receptor superfamily) binds, removes, and degrades collagenase-3, processes which are regulated by PTH. The intracellular pathway through which receptor-mediated degradation of collagenase-3 occurs has been defined. The loss of bone mass in microgravity may be due to an increased extracellular concentration of collagenase-3, resulting from an altered functioning of the collagenase-3 receptor. The current task seeks to test this hypothesis by identifying the domain on collagenase-3 which is responsible for binding to the collagenase-3 receptor.

During FY97, we successfully designed a system to express, purify, and radiolabel recombinant mouse collagenase-3. We have used this ligand in binding studies on normal osteoblasts, chondrocytes, and fibroblasts as well as on tumor cell lines (osteosarcoma and chondrosarcoma). We have shown that this ligand is internalized and degraded via the collagenase-3 receptor. We have also shown biological functionality through gelatin zymography.

To identify the ligand-binding site on the receptor, we have obtained (through a collaboration with researchers at Harvard) chimeric constructs of human collagenase-1 and mouse collagenase-3. Human collagenase-1 does not bind to the collagenase-3 receptor. Thus, insertion of different domains of the mouse collagenase-3 cDNA into the human collagenase-3 cDNA, followed by expression and radiolabeling of the chimeric protein will afford an elegant means to find the ligand binding domain. Thus far, we have subcloned and expressed two chimeric proteins and performed binding assays. We are currently using PCR to generate a new cloning site in two other chimeric cDNAs. Our early results are quite promising, and we expect to generate more data in the upcoming year.

This project seeks to understand a basic biological process which is important to bone physiology on Earth as well as in space. Our results could offer insight into the pathophysiology behind the loss of bone mass in microgravity and also under conditions of decreased bone loading on Earth (e.g., during prolonged bed rest). This research also has direct applicability to osteoarthritis and osteoporosis. Long-term benefits may include the development of pharmaceuticals which mimic the collagenase-3 receptor binding site in an effort to ameliorate bone pathology.

FY97 Publications, Presentations, and Other Accomplishments:

Barmina, O.Y., Fiacco, G.J., Walling, H.W., Omura, T.H., Lopez-Otin, C., Jeffrey, J.J., and Partridge, N.C. "Further characterization of the osteoblastic metalloproteinase scavenger receptor." (Abstract) *J. Bone Min. Res.*, 11 (suppl 1), S336 (1996).

Walling, H.W. "Regulation of the collagenase-3 receptor and its role in intracellular ligand trafficking in rat osteoblastic cells." Saint Louis University Graduate Student Association Poster Session (April 10, 1997).

Effects of Resistance and High-Impact Training on the Musculoskeletal System in Premenopausal Women

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UPN/Project Identification: 199-99-27-12
Initial Funding Date: 1996
FY 1997 Funding: \$22,000

Solicitation: GSRP 96
Expiration: 1999

Task Description:

Osteoporosis, characterized by bone loss leading to fractures, represents a significant health concern in this country. Aging accounts for bone loss of approximately 1% per year beginning in the third decade. Women are at particular risk for osteoporosis due to an accelerated bone loss of 2.0-6.5% per year within the first five to eight years after menopause. The etiology of osteoporosis includes aging, hormones, nutrition, genetics, and mechanical loading via exercise. Of these factors, only increased mechanical loading has been shown to stimulate bone formation. Conversely, space flight, immobilization, and bedrest report significant bone loss as a result of disuse. Russian cosmonauts experienced losses in BMD of 0-10% at the lumbar spine, 1.3-11.4% at the femoral neck and 0.4-9.5% at the tibia after 4.5-6.0 months aboard an orbital space station (Oganov et al., 1992). Significant decreases in pelvis, femoral neck, trochanter, and calcaneus BMD after 131-312 days in space have also been reported. It is not certain whether bone lost during space flight can be fully recovered upon return to a 1G environment. Recently, Nishimura et al. (1994) observed a 4.6% decrease in lumbar spine BMD and 3.6% decrease in metacarpal BMD in nine subjects after 20 days of bedrest. The female subjects in this study exhibited a two-fold increase in calcium excretion while male subjects showed no change despite similar losses in BMD. These findings may indicate a gender-specific response of bone to the effects of weightlessness. Mechanistically, it is not clear whether the bone loss associated with microgravity is more a result of increased resorption or decreased formation, but the existing literature in animals in space flight support a disruption in formation (Wronski and Morey, 1983). In addition to decreases in bone mass, reductions in weight bearing activity result in loss of muscle mass and strength. Declines in muscle mass and strength are also a function of aging and may be associated with age-related changes in BMD. Similarly, decreases in muscle mass and strength associated with loss of weight bearing activity may be partially responsible for reductions in BMD with inactivity and weightlessness. The deleterious effects of weightlessness on the musculoskeletal system are several-fold. Loss of muscle strength and power could compromise functional capacity and task performance, particularly if the work demands are high. Loss of BMD and bone strength could increase risk of fracture and possibly lead to premature osteoporosis. These consequences apply to both the 0-G and 1-G environments. Hence, effective countermeasures for minimizing decrements in the musculoskeletal system in microgravity are needed. While exercise programs for maintaining cardiovascular function have been successfully employed in space flight, this type of exercise has been unable to preserve muscular strength and bone mass. An in-flight exercise program to deter muscle and bone loss is difficult to design and to study. A more feasible approach may be to develop an effective pre-flight training protocol specifically developed to adequately load the musculoskeletal system and result in increases in both BMD and muscular strength. Higher pre-flight levels of

muscular strength and BMD may afford the astronaut a reservoir for the loss experienced in-flight. Work capacity and performance would be less compromised during space-flight and physiologic decrements upon return to 1G would be less dramatic. While muscle mass might be preserved through in-flight resistance training, maintenance of bone mass is less promising due to the absence of ground reaction forces. This phenomenon is exemplified by our studies in gymnasts who experience significant spine and lower extremity bone gains in-season and decreases during detraining in summer months (LaRiviere et al., 1994). Data from gymnasts clearly exemplify the dynamic response of bone to increasing forces. We have observed increases of up to 8% over nine months followed by declines which are just under one-third of the gains (LaRiviere et al., 1994). These results demonstrate that bone mass of the lower extremities (proximal femur) responds to high forces with a change greater than would be expected from the literature. Thus, the key factor appears to be creating forces high enough to induce an osteogenic response. The proposed study is designed to increase forces generated by the spine and lower extremities using a combination resistance/high-impact training program. Specifically, we will observe the effects of such a training program on bone mineral density at the proximal femur, lumbar spine and whole body, muscle strength, muscle power, bone-regulating hormones, and biomarkers of bone metabolism in 50 mature premenopausal women over a 12-month training period. It is hypothesized that the forces achieved from the exercise protocol – squats, lunges, and jumping routines from increasing heights – will result in significant increases in lower extremity BMD (proximal femur and femoral mid-shaft) as well as significant increases in lean mass, strength and power. This unique program is based on the force equation, $F = ma$, such that increasing mass with weighted vests and increasing acceleration from jumping off different box heights will effectively increase forces transmitted to the musculoskeletal system. The extended duration of the program is designed to allow for optimal increases in bone mineral density over time with exercise training. A six-month detraining period will follow the 12-month exercise training period. If the exercise program results in changes in any of the outcome measures, we will expect them to return toward baseline after six months of no training. Furthermore, an additional 25 women who will not participate in the exercise training, but will be tested at the same time intervals as the exercise group, will serve as controls.

The time period from October - December 1996 was spent in preparation for the upcoming study, and included pilot testing and reliability testing for all outcome measures. In addition, the project was successfully proposed to the doctoral dissertation committee in December.

Recruitment for participants began in January 1997. By February, fifty women from the Corvallis area and nearby communities had volunteered to participate in the exercise training program. In order to establish baseline rates of change on all outcome measures each woman was tested at the beginning and end of a six-month observation period which began in March of 1997 and was completed in early September. An additional cohort of women from the same geographic area was recruited in August of 1997 to serve as a control group. The control group was matched to the exercise group on the measures of right hip bone mass, age, height, and weight. The control group was tested on the same outcome measures as the exercise group in September. Control women will continue to be tested at the same six-month intervals as the exercise group, but will not participate in the exercise training.

The 12-month exercise training program began on September 27, 1997 and will be completed on September 25, 1998. Women are currently attending 3 training sessions per week, each session lasting 45-60 min. The exercise group will be tested in April 1998 (midway in training), September 1998 (when exercise training ceases), and in April 1999 for six-month follow-up testing after completion of the exercise training program. The control women will be tested over the same six-month intervals.

Osteoporosis, characterized by bone loss leading to fractures, represents a significant health concern in this country. Over 1.5 million fractures occur annually in the U.S. (Melton, 1994). Fractures in later life are associated with an increase in mortality and morbidity. Over 70% of all fractures in persons older than 45 yrs of age are attributed to osteoporosis, and women are more likely to fracture than men. Aging accounts for bone loss of approximately 1% per year beginning in the third decade. Women are at particular risk for osteoporosis due to an accelerated bone loss of 2.0-6.5% per year within the first five to eight years after menopause. The etiology of osteoporosis includes aging, hormones, nutrition, genetics, and mechanical loading via exercise.

The primary approach to preventing or delaying the onset of osteoporosis is to build bone mass prior to menopause, either via nutrition and/or exercise. Of these two strategies, only increased mechanical loading has been shown to stimulate bone formation after longitudinal growth has ceased. The exercise intervention program in the proposed study is designed to specifically build bone mass via increased ground reaction forces generated from jumping exercise and via increased mechanical strain generated from weight-bearing lower body resistance exercise. If this program successfully builds bone mass in this population, these women may have reduced their risk for osteoporosis and related fractures in later life.

The Role of Electrical Events in Controlling Differential Elongation and Gravitropism in the DEZ

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Funding:

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Solicitation: not available

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FY 1997 Funding: \$22,000

Task Description:

A specialized population of cells in roots called the distal elongation zone (DEZ) initiates differential elongation responsible for gravitropic curvature. Gravitropism proceeds in the DEZ even in the absence of an auxin gradient, provoking the question, "What signal is responsible for controlling differential growth in the DEZ?" Gravitropism induces changes in membrane potentials of root tip cells and changes in surface potential profiles along the axis of the root. Given these electrical events, and given the growth response of roots exposed to an electric field, this research addresses the hypothesis that DEZ cell expansion is regulated by electrical signals.

Although the project is in its early stages, progress has been made in characterizing the gravitropic response of *Arabidopsis* roots to the anion channel inhibitor NPPB. NPPB delays or inhibits gravitropic curvature in roots of *Arabidopsis* when applied at a concentration of 35 μM . This inhibition is not due to a general inhibition of elongation, since roots treated with NPPB continue to elongate at an average rate 65% that of control roots. In treated roots that showed gravitropic curvature, the lag time was increased and the rate of curvature was decreased relative to control roots. The anion channel inhibitor 9-anthracenecarboxylic acid (9-AC) is also a potent inhibitor of root gravitropism in *Arabidopsis* without causing significant inhibition of elongation. Current efforts are aimed at identifying the region of the root affected by these anion channel blockers. This descriptive characterization will form the basis of a model to be tested using electrophysiology.

As nonmotile organisms, higher plants respond to a variety of environmental signals by modulating growth magnitude or direction. Some environmental signals influencing plant development and growth include light quality, light quantity, touch, water availability, and gravity. While it is clear that each of these signals is perceived by the plant by different mechanisms, it is likely that the differential growth response of the plant to these signals may be accomplished by similar mechanisms. Therefore, using plant responses to gravity as a model system, we seek to understand the underlying motor mechanism driving differential growth in the plant root. The information learned from these kinetic and electrophysiological studies can be applied to growth responses occurring in other plant organs as a result of different environmental stimuli.

FY97 Publications, Presentations, and Other Accomplishments:

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NOTE: This NSCORT represents ten individual tasks with ten principal investigators.

Funding:

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Initial Funding Date: 1993	Expiration: 1998
Students Funded Under Research: 13	Post-Doctoral Associates: 5
FY 1997 Funding: \$1,130,000	

Task Description:

The objective of the NASA Specialized Center of Research and Training (NSCORT) at the University of Texas Southwestern Medical Center at Dallas (UTSWMC) is to advance space life sciences and integrative physiology through multidisciplinary research efforts that focus on the physiological adaptation to microgravity. Collaborative links have been formed between established scientists who are working at various levels with different organ systems but share the goal to define the mechanisms that underlie the responses to changing physiological loading conditions. The central theme is disuse atrophy as it occurs in microgravity and affects the musculoskeletal and cardiovascular systems.

The NSCORT at UTSWMC has a solid base of a strong institutional commitment to biomedical research. The campus environment provides access to a wide range of scientific expertise and facilities. Many of the NSCORT investigators have a well-documented long-standing interest in integrative physiology, and a long history of participation in NASA life sciences activities that range from ground-based research and space flight experiments to service on NASA advisory groups.

Section I on cellular and molecular mechanisms (principal investigators Drs. DeMartino and Radford) examines processes that are likely to be of general importance and mediate adaptations to changing physiological demands in multiple biological systems. The two primary areas of investigation are regulation of intracellular protein

degradation in skeletal muscle, and control of myocardial contractile performance. Section II on mineral metabolism (Dr. Pak) explores the mechanisms that are involved in bone loss and hypercalcuria as induced by immobilization or exposure to microgravity and its prevention. Section III on skeletal muscle structure and function has three components studying (a) human inborn defect of oxidative metabolisms (Dr. Haller), (b) substrate regulation in skeletal muscle in disuse atrophy (Dr. Bertocci), and (c) changes in muscle fiber type, water content, and perfusion during unloading (Dr. Fleckenstein). The last two projects make extensive use of magnetic resonance imaging and spectroscopy. Section IV is devoted to cardiovascular physiology, specifically to human cardiovascular regulation during changes in posture, including prolonged bedrest. Project (a) examines the role of cardiac mechanics in orthostatic intolerance (Dr. Levine); project (b) deals with the regulation cutaneous blood flow (Dr. Crandall); and project (c) studies baroreflex regulation of arterial blood pressure following simulated microgravity (Dr. Raven). Section V (Dr. Blomqvist) is devoted to space flight experiments (supported by separate NASA and NIH grants and contracts) and mathematical modeling of cardiovascular physiology at microgravity. A new pilot project (Dr. Victor) examines the role of neuronal nitric oxide in blood pressure regulation.

Training in integrated physiology is provided at multiple levels ranging from summer fellowships for high school students to support of formal graduate school education to post-doctoral research fellowships. The NSCORT and its investigators have had an important role in the development of a new graduate program (Ph.D.) in Integrative Biology within the core unit of the graduate school at UT Southwestern, the Division of Cell and Molecular Biology.

DeMartino et al. (Section I:1) have made significant progress defining the biochemical mechanisms of intracellular protein degradation and the physiological regulation of this process, specifically to determine the structure and function of the proteasome and its activators and inhibitors. Radford et al. (Section I:2) have examined tissue-specific subunits that may regulate the activity of cardiac cytochrome oxidase examining cardiac function. They have successfully generated mice that carry a mutation in the gene encoding (VIaH subunit). Radford et al. have also developed MR imaging and spectroscopy (^{31}P and ^{13}C) systems that provide detailed information on contractile performance, energy metabolism, and substrate utilization in beating isolated transgenic mouse hearts.

Pak et al. (Section II) are currently conducting a randomized comparison between alendronate and a placebo, based on a 3-week period of bed rest. Previous 12-week studies have mapped the effects on bone metabolism of bed rest alone. The results suggest that bedrest suppresses parathyroid hormone secretion and $1,25\text{-(OH)}_2\text{D}$ synthesis and reduces intestinal Ca absorption. Urinary calcium increases from a rise in renal filtered load and attenuation of PTH-dependent augmentation of renal tubular reabsorption. Hypercalcuria increases the risk of renal stone formation. The unit has also developed ultrasound critical angle reflectometry as a noninvasive means of assessing bone density and also established new approaches to the analysis of mRNA in human bone tissue.

Haller et al. (Section III:1) have continued to study inborn errors in skeletal muscle metabolism as models of deconditioning and adaptation, including the cause and consequences of impaired oxidative phosphorylation linked to impaired muscle glycogenolysis, substrate delivery as a function of muscle oxidative demand in phosphofructokinase deficiency, and adaptations to physical training in muscle glycolytic defects. Recent studies have demonstrated that sympathetic activation during exercise in metabolic myopathies is independent of muscle pH, and that potassium is a possible mediator of the exaggerated sympathetic activation during exercise in McArdle patients. Bertocci et al. (Section III:2) have developed a novel methodology that uses administration of ^{13}C labeled substrates in rats to monitor substrate utilization using high resolution magnetic resonance spectroscopy. This technique is combined with hindlimb suspension to examine the effects of disuse atrophy. Fleckenstein et al. (Section III:3) have explored the use of several magnetic resonance techniques, including proton imaging, to monitor the effects of deconditioning on skeletal muscle properties.

Levine et al. (Section IV:1) have continued to explore the effect of bed rest on cardiovascular structure and function. Regulatory responses demonstrated that the human heart atrophies during prolonged bed rest. They

have shown that the well-documented bed rest-induced decrease in left ventricular end-diastolic volume is associated with a loss of myocardial mass and increased diastolic stiffness and significant changes in the opposite direction after physical training. Bed rest also affects the regulation of the cerebral circulation. A novel approach (transfer function analysis) to the characterization of the relationship between systemic and cerebral circulation revealed increased coherence, i.e. suggesting less effective autoregulation after bed rest. Limb arterial compliance, particularly in the leg, is also decreased after bed rest. There is also an attenuated increase in sympathetic nerve activity (as demonstrated by microneurography) during LBNP. Crandall et al. (Section IV:2) have focused on neural regulation of the microcirculation. Studies in human subjects have demonstrated cutaneous vasoconstriction and impaired thermoregulatory capacity after a 2 week period of bed rest. Crandall et al. have also demonstrated that (1) cardiopulmonary baroreceptor unloading during hyperthermia attenuates cutaneous vasodilator responses, and (2) β -adrenoceptor activation is not responsible for cutaneous active vasodilatation during heat stress. Raven et al. (Section IV:3) have continued their work on the interactions between cardiopulmonary and arterial baroreflexes. Both have been shown to have increased responsiveness after bed rest. Cardiopulmonary baroreflexes are attenuated in highly fit subjects.

Blomqvist et al. (Section V) have completed a study (with Dr. R.J. White) using mathematical modeling to examine the apparent paradox of large heart size and low filling pressure in humans early on orbit. Work on the derivation of beat-to-beat stroke volumes from continuous noninvasive records of finger blood pressure has continued. Members of the NSCORT have also continued their work on two major current space flight experiments (NeuroLab and Mir) on cardiovascular neurohumoral regulation.

Victor et al. (Section VI) are performing a pilot project on the role of neuronal nitric oxide in blood pressure regulation, including studies in humans, rats, and mice.

The Blomqvist section (Section VII-Knowledge Transfer) has contributed to the establishment of a new graduate program in Integrative Biology within the principal graduate school unit at UTSWC, the Division of Cell and Molecular Biology. NSCORT summer programs have involved 9 undergraduate students. Two graduate students have received full support from this section. Members of the NSCORT have participated in two international workshops (sponsored by CNES and by DLR) on future directions of life sciences space research and the proceedings from the workshop on cardiovascular science in space, held at UT Southwestern have been published. Dr. Blomqvist was a lecturer at the NASA Life Sciences Training Program at Kennedy Space Center and at the summer session of the International Space University at Rice University, Houston.

An improved understanding of the mechanisms that enable living organisms to adapt to microgravity and re-adapt to Earth gravity is an important NSCORT goal. Increased knowledge of the mechanisms that are involved in cardiovascular and musculoskeletal adaptation to microgravity will provide an important contribution to space medicine and is a prerequisite for adequate support of prolonged space travel. Detailed information on these mechanisms is also likely to have important implications for clinical medicine.

Studies on the cellular and molecular level within the NSCORT are providing new data on fundamental mechanisms that control the structure and function of the cardiovascular and musculoskeletal systems. New information relevant to the prevention of structural and functional losses in space can find immediate applications on Earth, (i.e., by helping to define new strategies to prevent cardiovascular dysfunction and loss of skeletal muscle mass following prolonged bedrest). Our NSCORT unit on mineral metabolism has developed new and effective methods to prevent mineral loss and stone formation in the urinary tract, methods applicable both to space and general medicine. Studies of cardiovascular dysfunction following actual and simulated microgravity have provided new insights into the mechanisms involved in orthostatic hypotension, an important condition that is commonly encountered in general medical practice. New findings include information on the effects of simulated microgravity (bedrest) on the mechanical properties of the heart and blood vessels and on the control of blood flow to the brain. The work performed within the NSCORT section on skeletal muscle metabolism and function also has the potential to produce new concepts and techniques that may become relevant to clinical medicine.

FY97 Publications, Presentations, and Other Accomplishments:

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NSCORT: Radiation Health

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NOTE: This NSCORT represents six individual tasks with six principal investigators.

Funding:

UPN/Project Identification: 199-93-17-07
Initial Funding Date: 1992
Students Funded Under Research: 13
FY 1997 Funding: \$1,000,039

Solicitation: NRA
Expiration: 2002
Post-Doctoral Associates: 1

Task Description:

The first major goal of the proposed Center is to conduct basic and applied radiobiological research with HZE (high atomic number, Z, and high energy, E) particles that is directly applicable to the assessment of the radiation risks associated with extended manned space missions. Proper knowledge of these risks will allow NASA to determine the measures needed to protect human beings against the effects of ionizing radiations in space. Basic research efforts will focus on several different but highly interactive approaches in order to provide critical information needed to assess the risks of carcinogenesis from exposure to protons and HZE particles during space travel. Theoretical studies will address track structure and quantitative estimation of initial DNA damage for all HZE particles of interest. Experimental studies of enzymatic DNA repair processes will extensively characterize repair by normal human cells as measured by four different end points and then compare the repair responses of rodent and human cells in order to assist in the extrapolation of mutagenesis, transformation, and carcinogenesis data from rodent systems to humans. Comparative mutagenesis studies will be conducted with two different cell systems, one human and one rodent, to evaluate mutational risks under different genetic constraints and to determine the effect of genetic linkage and of DNA repair capacity on the types of mutations recovered. Transformation of mouse mammary epithelial cells will be quantified using an *in vitro* focus assay, and the ability of these foci to undergo neoplastic progression in the mouse in different tissue environments will be investigated. Applied research will be directed toward assessing the risk of radiation cataractogenesis by conducting a retrospective analysis of cataractogenesis in human patients treated therapeutically at LBL with helium ions and comparing these data to the extensive data base available for experimental animal cataractogenesis. Finally, extrapolation procedures for human risk assessment will be explored to facilitate relating results across species and from high to low doses/fluences.

The other major goal of the Center is to promote education and training in broad areas of space radiation studies but with special emphasis on the biological effects of HZE particles. The Department of Radiological Health

Sciences at Colorado State University will be the home of the educational program. Most of the research involvement of students pursuing graduate studies, and the training of postdoctoral candidates, will be at LBL.

The major objective of the NSCORT is to continue to advance research and training of students in Space Radiation Health through an established consortium between the Department of Radiological Health Sciences at Colorado State University (CSU) at Fort Collins and the Life Sciences Division at the Lawrence Berkeley National Laboratory. The training and research center has been operating since 1992.

Student Training

In FY'97, two students received their Ph.D. degrees and another student is expected to complete it in December 1997. Three new students have been added to the program. In addition to students who are directly supported by NSCORT funds, one student from U.C. Berkeley (Bioengineering Department) has also joined as a Ph.D. candidate under the mentorship of Mary Helen Barcelos-Hoff, who is one of the principal investigators in the NSCORT project. Now that we have a Web page dedicated to NSCORT, we are receiving a lot of inquiries from students all over the country and abroad. In the future, we expect to increase the number of students only by four or five and this limit is set by the available budget in the NSCORT project.

Research

PROJECT 1: Theoretical Modeling of DNA Damage and Cellular Responses (Chatterjee)

Progress: Evidence has been provided in support of a previously proposed zig-zag ribbon rather than a solenoidal configuration as the basic structural organization of 30 nm chromatin fiber. This evidence is obtained by comparing theoretical predictions of damage to DNA by ionizing radiation with results of recent experimental measurements by Björn Rydberg. For the first time, it has been demonstrated that radiation induced damage analysis provides a novel approach to obtain information on chromatin structure *in situ*. Theoretical calculations involving chromatin, modeled either as a 30 nm diameter solenoid or as a 30 nm zig-zag ribbon, predict the induction by ionizing radiation of substantial amounts of small-sized single- and double-stranded DNA fragments. Furthermore, the predicted size distributions of the fragments exhibit features characteristic of the particular chromatin structure considered. The experimental measurements of Rydberg, using human fibroblast cells and several different types of ionizing radiation, differ significantly from the calculated distributions for the solenoid model. When the calculations are made using zig-zag ribbon models, the theoretical distributions reproduce all the experimentally observed peaks. This agreement provides evidence for the zig-zag model of chromatin fiber.

PROJECT 2: DNA Repair and the Early Development of Chromosomal Changes (Cooper, Bedford)

Progress: Induction of DNA double-strand breaks (dsbs) in mammalian cells is dependent on the spatial distribution of energy deposition from the ionizing radiation. For high LET particle radiations, the primary ionization sites occur in a correlated manner along the track of the particles, while for X-rays, these sites are much more randomly distributed throughout the volume of the cell. It can therefore be expected that the distribution of dsbs linearly along the DNA molecule also varies with the type of radiation and the ionization density. Using pulsed-field gel and conventional gel techniques, we measured the size distribution of DNA molecules from irradiated human fibroblasts in the total range of 0.1 kbp-10 Mbp for X-rays and high LET particles (N ions, 97 keV/ μ m and Fe ions, 150 keV/ μ m). On a mega base pair scale, we applied conventional pulsed-field gel electrophoresis techniques such as measurement of the fraction of DNA released from the well (FAR) and measurement of breakage within a specific NotI restriction fragment (hybridization assay). The induction rate of widely spaced breaks was found to decrease with LET. However, when the entire distribution of radiation-induced fragments was analyzed, we detected an excess of fragments with sizes below about 200 kbp for the particles compared with X-irradiation. X-rays are thus more effective than high LET radiations in producing smaller fragments. We determined the total induction rate of dsbs for the three radiations based on a quantitative analysis of all the measured radiation-induced fragments and found that the high LET particles were more

efficient than X-rays at inducing dsbs, indicating an increasing total efficiency with LET. Conventional assays that are based only on the measurement of large fragments are therefore misleading when determining total dsb induction rates of high LET particles. The possible biological significance of this non-randomness for dsb induction is discussed.

PROJECT 3: Mutagenesis and Chromosomal Alterations (Kronenberg, Waldren)

Progress: We have determined the number of mutants and the types of mutations induced by $^{137}\text{Cs-}\gamma$ and by HZE-Fe (^{56}Fe [600 MeV/amu, LET = 190 KeV/ μm]) in standard A_L human hamster hybrid cells and in a new variant hybrid, A_L -179. We found that HZE-Fe was more mutagenic (about 1.6 fold) than $^{137}\text{Cs-}\gamma$ per unit dose, but slightly less mutagenic per mean lethal dose, D_{01} , at both the S1- and *hprt* loci of A_L cells. But, nine fold more (28% vs 3%) of the S1- mutants induced by HZE were complex than those induced by $^{137}\text{Cs-}\gamma$ rays. $^{137}\text{Cs-}\gamma$ -induced twice as many S1- and *hprt* mutants in A_L -179 as in A_L . Nine fold more of these S1- mutants in A_L -179 were complex than in A_L .

PROJECT 4: Role of the Microenvironment in the Radiation Response of Epithelial Cells (Barcellos-Hoff, Blakely, and Gillette)

Progress: High-LET radiation has unique physical and biological properties compared to sparsely ionizing radiation. Recent studies demonstrate that sparsely ionizing radiation rapidly alters the pattern of extracellular matrix expression in several tissues, but little is known about the effect of heavy-ion radiation. This study investigates densely ionizing radiation-induced changes in extra-cellular matrix localization in the mammary glands of adult female BAB/c mice after whole-body irradiation with 0.8 Gy 600 MeV iron particles. The basement membrane and interstitial extracellular matrix proteins of the mammary gland stroma were mapped with respect to time postirradiation using immunofluorescence. Collagen III was induced in the adipose stroma within 1 day, continued to increase through day 9 and was resolved by day 14. Immunoreactive tenascin was induced in the epithelium by day 1, was evident at the epithelial-stromal interface by day 5 - 9, and persisted as a condensed layer beneath the basement membrane through day 14. These findings parallel similar changes induced by γ irradiation but demonstrate different onset and chronicity. In contrast, the integrity of epithelial basement membrane, which was unaffected by sparsely ionizing radiation, was disrupted by iron-particle irradiation. Laminin immunoreactivity was mildly irregular at 1 h postirradiation and showed discontinuities and thickening from days 1 to 9. Continuity was restored by day 14. Thus high-LET radiation, like sparsely ionizing radiation, induces rapid remodeling of the stromal extracellular matrix but also appears to alter the integrity of the epithelial basement membrane, which is an important regulator of epithelial cell proliferation and differentiation.

Ionizing radiation plays a very important role in our everyday life. The technological and medical applications of radiation and radioactivity have a long history. In addition to these benefits, ionizing radiation can be hazardous to humans, both on ground and in space. Hence, radiation can be beneficial as well as risky. It is extremely important that we understand at a fundamental level, the effects of ionizing radiation on living cells, tissues, and organs. Research in this project addresses many questions related to these understandings through basic research. Much of the investigation is focused towards human cancer-induction as well as cure of this disease. As far as induction of cancer is concerned, the findings of the research are equally applicable on Earth and in space.

In addition, this research also addresses radiation-induced cataractogenesis. The results of our study, which quantitatively have emphasized the vulnerability of the lens epithelial layer for the risk of radiation-induced cataract, have drawn the attention of the radiation oncologists at the new proton therapy facility at the University of California at Davis. Novel treatment plans have been initiated for uveal melanoma patients using two ports with different azimuthal angles to deliberately spare the lens epithelium. As a result, 55 new proton patients have been added to our cataract follow-up study since May 1994. These patients will add information of cataract risk to low fluences of protons and allow a comparison with the data from the helium-ion treated patients.

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Task Description:

The underlying assumption of this Center is that ground-based studies combined with past (and future) space flight data will provide information to support models that approximate human response to contaminants and conditions in space habitats. The degree to which these models deviate from actual conditions in space will contribute to our understanding of the role of gravity, confinement, and radiation. Such models will make visible the pervasive but invisible role of space constraints like gravity and confinement in the human response to stress from contaminants. Indeed, at the most basic scientific level, the distinguishing feature of space environmental health is the study of the role of gravity and confinement in determining human health risks from chemicals, airborne particles, microorganisms, and viruses. Physical phenomena that depend on the force of gravity, weight, density, convection, sedimentation, and hydrostatic pressure definitely play a role in the vestibular, musculoskeletal, and endocrine systems and may play a role in human risk from environmental contaminants. Thus, airborne particles, in the absence of sedimentation and convective flow, persist for longer periods in the atmosphere. The human host, compromised by microgravity-related effects—reduced red cell mass, calcium loss, muscle atrophy, diminished immune response—may respond differently to toxic or infective

stress than in normal gravity. Confinement may also play a critical role in these processes and may affect human neuroresponses.

The specific goal of this Center is to conduct ground-based research to minimize health risks so that the survival and productivity of astronauts are not compromised by contaminants or other environments in the spacecraft, and to train investigators in life sciences, medicine, engineering, and the physical sciences in this new subdiscipline of space environmental health. This Center will focus on two major sources of health risks: airborne chemicals and particulates, and recycled water contaminants. In addition, the Center promotes generic projects for the assessment of risk and the development of modeling tools to assess the environmental health state of the habitat and crew during long-term space flight.

The health of astronauts in habitats beyond Earth has always represented a challenge to the disciplines of environmental health and environmental medicine. The space habitat is similar to a small tight building. Both chemical and microbial contaminants have the potential to accumulate in a habitat of limited volume and resource turnover. Microgravity and the absence of convection currents will produce unusual behavior of suspended particles and of fluid and mass transport in the habitat and life support systems.

The response of astronauts to both toxic chemicals and invasive biota may differ from the Earth-based situation. The astronaut's physiological state in microgravity is altered, and is not totally understood. In addition, there is the potential for additional stress from solar and cosmic radiation. Crews will also be exposed to a diverse range, concentration, and type of potential contaminants. Exposures will be either chronic (low level) or acute (high level) in the case of accidents. Effects of concern include short- and long-term health effects and those that will affect performance and productivity of the astronauts.

While immediate emphasis is being placed on Earth orbital systems and, specifically, the health and productivity of astronauts on prolonged stays on the International Space Station (ISS), the research infrastructure is being established to supply the necessary guidance for the design of spacecraft and space habitats for missions to the Moon and Mars, planned for the next century. Of special note is the fact that such missions will be of much greater duration than those for Earth orbital missions.

The *modus operandi* for the Center has been to use a systems approach to link research activities. We believe this combination of human health risk assessment based on biological principles with systems engineering is a unique approach to problems in space environmental health made possible by the collaboration of life sciences and space engineering faculty in this consortium.

Risk assessments are made utilizing all available information on dose-response and human exposure to characterize the risk to human health. We have developed an extensive database on space environmental health issues and spacecraft contaminants as a first step in risk assessment. This database has been useful in identifying space and analogous environmental health information relevant to ongoing research projects. In addition to the published scientific literature on space hazards, our consortium has access to relevant NASA in-house reports that are useful in hazard identification. We also try to use experience gained from surrogate situations such as nuclear submarines, deep sea diving submersibles, and Antarctic bases.

In all phases of these studies, we attempt to take into account the special modifying factors unique to the astronauts' environment - especially exposure to microgravity and radiation. Research supported by the NSCORT is specifically limited to the ground-based environment so the effects of microgravity on contaminant behavior and astronaut response must be modeled on the basis of available theory and evidence. The projected effects of radiation also depend mainly on extrapolation and modeling, but the possibility does exist for doing direct studies of combined exposure to contaminants and radiation.

FY 97 Report, University of Rochester Team

The research conducted by this NSCORT at the University of Rochester is carried out by teams of biological scientists and engineers. This portion of the Fiscal Year 1997 report, therefore, will summarize team accomplishments rather than individual work.

Inhalation Risk Team: The major discovery by this team is that ultrafine particles, liberated by the overheating of polytetrafluoroethylene (Teflon™), are highly toxic to the lung. Experiments clearly implicated ultrafine particles as opposed to other substances released from Teflon. The overheating of Teflon, such as might occur with electrical insulation, produces large numbers of ultrafine particles and gaseous materials. Studies on rats revealed that only the ultrafine particle component was able to induce inflammation. The inflammatory response is mediated by cytokines generated by lung macrophages. A second series of experiments revealed that brief exposures to ultrafine particles can protect against longer exposures that otherwise would induce inflammation. The brief pre-exposures upregulated antioxidant and anti-inflammatory defense mechanisms.

Human Performance Risk: This team was established because any deleterious effects on astronaut performance could critically affect manned space missions. Toluene is present in spacecraft due to outgassing from plastics and other polymeric materials. Generally, toluene is found at concentrations higher than other volatile organic solvents. Studies were conducted on six healthy volunteers who were exposed, in six-hour periods, to toluene at the industrial threshold limit value of 100 ppm, an air concentration hitherto believed to be non-toxic. No effects were found on lung function, but certain aspects of performance were adversely affected. As determined by a one-hour complex performance test (SYNWORK), the composite score was about 10% lower in the last hour of exposures to toluene as compared to controls. Differences in performance between air alone and toluene were greatest after exercise. Unfortunately, due to cessation of funding, the team has not been able to conduct tests for synergistic effects of additional stresses such as sleep deprivation and infection, stresses that may well be important in space missions.

Water Recycling Team: This team constructed a water recycling test-bed to study the growth and persistence of viruses in recycled water and to test the effectiveness of disinfection techniques. MS-2 strain coliphage was chosen as the model virus, and iodine as the disinfectant. Analytical methods were refined to identify and measure iodine disinfection products. The modified method allows the measurement of idoform which is likely to be the principle disinfection product. The formation of biofilms has been found to play a key role in the persistence of viruses and resistance to infection. Studies have been conducted on the role of molecular size and change on biofilm absorption of organic matter and on the effect of ozonation of the sorption of natural organic matter by biofilm.

Quantitative Assessment Team: As its main purpose, this team sought to quantify health risks to astronauts based on the research findings of this NSCORT. Thus, as the NSCORT teams developed new data, the role of the Risk Assessment Team has grown in importance. A seven-step model was completed for the selection and general placement of sensing devices for airborne contaminants in a predefined space habitat. The latter was assumed to take the form of three interconnected Space Station modules. The contaminants were chosen to be hydrazine, ammonia, and carbon monoxide. The full model was reported in a student thesis completed in December, 1996. Specifically, utilizing the designed contaminant monitoring system reduced the simulated costs associated with severe contamination events by 50%.

Training and Outreach Program: Two masters and two Ph.D. students have graduated, and have now taken positions in academia or in space-related industries. Four undergraduate minority students were given hands-on laboratory experience in the summer months of 1996. The outreach program has been responsible for presentations in high schools, science museums, and to civic audiences such as retired professionals and veterans. In fact, the outreach program is receiving increasing requests for educational presentations and displays.

FY 97 Report: University of Colorado Team**Project Synopsis: Atmospheric Hazard Identification and Environmental Monitoring**

Safe air is a vital environmental requirement for crew members during space missions. Grossly contaminated air can cause incapacitation and death. Slightly contaminated air can cause subtle health and performance decrements. Contamination can be caused by long-term effects such as the accumulation of biological toxins or by an accidental release such as the release of fine particles and/or toxic gases due to the thermal degradation of fluoropolymers in electrical insulation (such as Teflon™) associated with the failure of electronic equipment.

The main objective of this research project was to develop an intelligent monitoring system capable of detecting and diagnosing contaminant emission concentrations and methods of measuring crew performance, impairment, or safety risk therefrom. To do this, we are developing an accurate model of contaminant release and transport, a detection system that uses both process information and sensor information, an optimal sensor selection procedure, a technique for determining the location and capacity of release events, and applied technologies for measuring toxin/stress induced performance degradation.

This project investigated the feasibility and expediency of running a model of contaminant introduction, dispersion, and removal in real time, and uses it as the basis for the solution of problems of monitoring air quality, fault detection, and fault diagnosis. The results of this investigation were recommendations on the development of prospective air revitalization systems for human-occupied spacecraft and/or the prescription of astronaut regimens of exercise, oxygen-breathing, and medication so as to avoid astronaut endangerment or inability to perform the mission. Life sciences leaders in NASA are concerned about committing astronauts' lives to long-term space missions such as the Mars trip until these and other crew risk/performance problems have reliable solutions.

This research on modeling, monitoring, and fault diagnosis of spacecraft air contaminants can also be applied to such terrestrial venues as large buildings, airplanes, submarines, subways, and surface ships.

Hypotheses

To solve the problem of detecting, evaluating, and remediation of spacecraft air contaminants, we worked under the following hypotheses: safe, effective missions require the ability to model the release and transport of contaminants in a spacecraft, to develop a sensor selection procedure, to develop an intelligent monitoring system using both process knowledge and measurements, and to perform fault diagnoses and valid evaluations of health risks.

Organization of Research Projects.

The NSCORT research projects were developed and executed according to the risk assessment paradigm first put forward by the National Research Council (NRC) in 1983. The identification of chemical, particulate, and microbial hazards in atmosphere and water is the first step in risk assessment. In the first year of funding, the NSCORT concentrated on this step. The aerospace experience of Martin Marietta and the scientific contacts of the Center for Space and Advanced Technology, Fairfax, Virginia allowed us to identify certain space contaminant hazards and to allocate priorities for research projects. Establishing an Environmental Toxicology Database was a major step in the hazard identification process. In addition, the original NASA Request for Proposal (RFP) asked for the development of contaminant concentration modeling and risk assessment methodologies so that NASA would ultimately have improved tools for space habitat design and human space mission planning.

The subject work, which stemmed from advances made in the research conducted in the NSCORT has encompassed the following activities:

1) The development of a fault-detection mathematical model utilizing Kalman filters was accomplished by Professor Fred Ramiriz and graduate student Michael Skliar. This research culminated in Mr. Skliar's doctoral thesis "State Estimation and Fault Diagnosis for Distributed Parameter Transport Processes With Application to Air Contamination Control," and the award of a doctoral degree to Dr. Skliar in August of 1996. This work is being further developed in the thesis research of graduate student Anand Narayan, "Modeling, Estimation, and Fault Diagnosis of Air Contaminants in Spacecraft."

2) The development of an optimum sensor allocation model based upon the utilization of Korbitz filters by graduate student Gerald Smith under the direction of Professor George Morgenthaler. This research resulted in Mr. Smith's doctoral thesis "Sensor Selection Methodology for Detecting and Monitoring Toxic Concentrations of Airborne Contaminants in a Space Habitat," and the award of a doctoral degree to Dr. Smith in December, 1996.

Dose-response assessment involves complete toxicological evaluation. The major health endpoint of concern is deterioration in the cognitive or physical performance of astronauts. Lung damage, either temporary or permanent, could directly affect physical performance, and indirectly, psycho-physiological capacity. For example, the inhalation of toluene has the potential to depress the central nervous system. Likewise, deterioration of the quality of reprocessed water is life and mission threatening. The immune and hematopoietic systems may be especially vulnerable to contaminants, since they are the site of interaction of several other stresses associated with space flight, including microgravity, radiation, and stress. We therefore decided to develop an interdisciplinary team of basic and clinical scientists in this area.

Studies on exposure assessment focused on the potential for exposure with a view to a quantitative prediction of the doses of toxic compounds astronauts might receive during long-term space missions. Exposure assessment therefore became an integral part of our two major research areas: inhalation risk and water quality. For example, the thermodegradation area had projects on the chemical products of Teflon™ degradation and on the airborne transport and accumulation of these contaminants in microgravity. The water recycling studies were concerned mainly with exposure assessment, including the production and growth of contaminants, the identification of contaminants, and the survival of pathogens. A human biological monitoring component was also completed. This activity involved the analysis of human hair, urine, and saliva to measure the body burden of iodine compounds used as disinfectants.

Once we have determined the relationship between dose and response for a given contaminant and, having determined the probable exposure of the astronaut to the contaminant, we shall be in a position to characterize the health risks in real flight situations. Should such risks be sufficiently great to raise concerns about safety and mission-performance, the development of risk management procedures will be required. This was addressed for inhalation risks by the development of an Atmospheric Risk Assessment and Management Model.

The use of the models developed in this present work thus could be of direct operational use to those in NASA who are directly responsible for the well-being of space crew members. Such remediation uses might involve changes in the Space Maximum Allowable Concentration (SMAC) for the substance(s) in question; modifications to contaminant detection and removal systems; development of advanced mitigation and antidotal procedures; and possibly, modifications to mission plans or habitat designs (e.g., use of artificial gravity and a vigorous exercise regimen to mitigate bone mineral loss and muscle atrophy).

The NRC risk assessment paradigm allows the application of systems analysis to the broad topic of modeling, monitoring, and fault diagnosis of spacecraft air contaminants. For example, the need for antidotal therapy in the risk management phase will stimulate further work on the toxic mechanisms of action. An understanding of the effects of contaminants at the biochemical and cellular levels could provide a basis for the rational development of protective treatment procedures.

As is well known, major uncertainties exist in the present ability to interpret the dose-response data and the risk characterization data associated with the likely spacecraft atmospheric contaminants such as hydrazine,

ammonia, CO, CO₂, fluoro-hydrocarbons, etc. Some of these materials obey Haber's Law (i.e., concentration x exposure time = constant), but some do not. Also, when several contaminants are simultaneously present, yet none exceed their individual SMAC levels, we must also ask these questions: (1) Will the mixture of gases and/or particulates exceed human thresholds of safety and/or cause excessive performance impairment? and, (2) Will there be a chemical reaction between the contaminants, producing yet other contaminants which may have a more serious (or different) impact on astronaut safety and/or performance ability?

While it is necessary and desirable to continue the basic toxicological research that can answer the above questions, it is pragmatic and operationally useful to seek to identify means of quantitatively measuring the dose-response of humans to the contaminants likely to be present in a spacecraft environment. In this way, long-term human space exploration and utilization missions can be safely undertaken while we await development of a comprehensive dose-response knowledge base. This is a particularly attractive policy now because, with the advent of the International Space Station, the development and testing of pragmatic atmosphere containment astronaut protection systems is possible, and will enable human expeditions to visit Mars in the first quarter of the 21st century. This has been indicated to be a NASA goal.

With this goal in mind, two innovative instruments were identified that offered the potential to be developed as real-time, quantitative monitors of human performance impairment in the presence of toxic atmosphere substances, drugs, alcohol, or stress: (1) PACE™, a proprietary software/PC-based system for measuring reaction time and recognition time, developed by Professor German Nuñez (now at the University of Colorado, Boulder) and his colleagues at Florida Atlantic University; and, (2) the VeriFax Corporation's Impairometer, which is a special instrumented pen which measures accelerations in the X and Y directions and associated pressure in the Z direction, when a person writes his/her signature. It calculates the neuromuscular performance in terms of correlation coefficients of X and Y, X and Z, and Y and Z to detect impairment vs. earlier unstressed scores.

Our objective was to test the ability of prototype versions of these instruments to see if they could quantitatively measure the degree of impairment due to the presence of atmospheric contaminants and to devise an on-board spacecraft atmospheric control system. The system we envisioned would use the Ramirez/Skliar contaminant leak identification system, the Morgenthaler/Smith contaminant selection and sensor location strategy, and the above PACE™ and VeriFax impairment measuring instruments to establish a feedback contaminant control regulator built upon the NRC process.

During the Summer and Fall terms, 1997, over 30 students and faculty were tested for degradation of reaction/recognition times and/or deterioration of handwriting biometrics (smoothness) due to the impact of a variety of stressors. The funding for the testing of the capability of these devices and the publication of results was obtained as follows:

1) VeriFax: Dr. Ruth Shrairman and Mr. Alex Landau of VeriFax Corporation, co-inventors of the patented Impairoscope technology, demonstrated the ability of the Impairoscope to measure the performance improvement in Parkinson's patients upon administration of the patients' L-dopamine medication. These tests were conducted at the Colorado Swedish Hospital Neurological Research Center. Also, trials were conducted with nine volunteers who consumed alcohol. These latter tests were conducted at the University of Colorado's Alcohol Research Center. The VeriFax Impairometer successfully measured the degree of signature impairment vs. blood/alcohol ratio. Both of these tests were performed at VeriFax's expense and/or under NASA-SBIR funding. The VeriFax Corporation donated the time of Dr. Shrairman and Mr. Landau during the University of Colorado's Summer stress/impairment test program. The support funding for student Aaron Botello was supplied by the Western Alliance to Expand Student Opportunities (WAESO)/NSF. Student José Soto received support from the University of Colorado's Summer Minority Access to Research Training (SMART) program. Dr. Morgenthaler's time and some of the student support time associated with this Summer, 1997 activity were provided partially by the present NRA 93-OLMSA-07 and partially by residual FY'97 NSCORT funds.

2) PACE™: Dr. Nuñez's time was made available by the University of Colorado. The students (Aaron Botello and José Soto) and Dr. Morgenthaler received funding as noted above.

The results of the investigations were:

- 1) The PACE™ and the VeriFax Impairoscope instruments appeared to be effective in measuring the degree of impairment due to the contaminants and the neuromuscular deterioration stresses examined in our experiments. Admittedly, sample sizes were limited due to budgetary constraints. Stress levels were likewise limited due to the lack of a clinical test environment that was safe for more extreme stresses. Therefore, strong conclusions cannot be made at this time. However, we believe that the intriguing data collected during this effort provide favorable indications toward evolving these prototype instruments into reliable, practical clinical/mission operational devices.
- 2) Two publications have been accepted for the Space Technology & Applications International Forum (STAIIF-98) Conference (and Proceeding volumes) at Albuquerque, New Mexico, January 26-29, 1998: (a) Morgenthaler, G.W., R. Shrairman, and A. Landau, "VeriFax: Biometric Instruments Measuring Neuromuscular Disorders/ Performance Impairments;" and, (b) Morgenthaler, G.W., G. Nuñez, R. Shrairman, and A. Landau, "Investigation of PACE™ Software and VeriFax's Impairoscope Device for Quantitatively Measuring the Effect of Stress."

Training, Education and Outreach

As is documented in the bibliography and in prior Fiscal Year Reports, this NSCORT was very active in giving conference papers, K-12 outreach, and undergraduate and graduate training. Also, a database on Space Environmental Health was supplied to NASA. However, this activity formally ceased in late 1997.

Fiscal Year 1997 Report, Martin-Marietta Team, Center for Space and Advanced Technology Team and Harvard University

The research tasks are directly relevant to understanding certain human disease processes. The study on ultrafine particles has led to the hypothesis that such particles may contribute to human morbidity on Earth. Indeed, we now suspect that lung function in areas of air pollution such as in the large industrialized cities may in part be due to the inhalation of ultrafine particles. Such particles are not normally detected by the commonly used filters for airborne particulate pollutants. Thus, this project has given rise to a new approach to assessing the causes of lung damage from air pollution.

The studies on toluene have also given new insights into the Earth-based problem of indoor air pollution both in the workplace and in the home. People are increasingly finding themselves having to perform complex tasks in situations with multiple stresses. This study has already alerted the occupational medicine community that subtle effects of chemicals on complex performance tasks can and do occur at air levels of toluene hitherto believed to be safe. Future studies would have examined the combined effects of several stresses such as sleep deprivation, cold or flu infections, and exposure to airborne pollutants to mimic real life workplace conditions in an increasingly sophisticated work environment.

The persistence of viruses in drinking water remains a major public health concern especially in third world countries. Infant mortality can still reach appalling levels, even exceeding 50% in countries with poor sanitation. Our studies in water disinfectants and the formation of biofilms that hinder the disinfection process are directly related to these ground-based public health problems.

FY97 Publications, Presentations, and Other Accomplishments:

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NSCORT. Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology

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 Joan L. Huber; North Carolina State University
 Steven C. Huber; North Carolina State University
 Gloria K. Muday; Wake Forest University
 Dominique Robertson; North Carolina State University
 Ronald R. Sederoff; North Carolina State University
 William F. Thompson; North Carolina State University
 Edward B. Tucker; Baruch College, City University of New York
 Ross W. Whetten; North Carolina State University
 Eric Davies; North Carolina State University

NOTE: This NSCORT represents 12 individual tasks with 12 principal investigators.

Funding:

UPN/Project Identification: 199-93-17-14
 Initial Funding Date: 1996
 Students Funded Under Research: 15
 FY 1997 Funding: \$999,789

Solicitation: 94-OLMSA-04
 Expiration: 2000
 Post-Doctoral Associates: 7

Task Description:

This program in gravitational biology involves 9 faculty members from North Carolina State University, Wake Forest University, Baruch College, and the Dynamac Corporation through the Kennedy Space Center. The overall goal is to study calcium as a central focal point in the gravity response. The group uses an integrated molecular, cellular, and physiological approach to plant gravitational biology.

The precise modulation of calcium homeostasis will be achieved using transgenic technologies and monitored using sophisticated imaging techniques to verify the specificity and extent of transgenic expression. These efforts, in combination with our expertise in local and long-distance signalling, will make a major contribution to understanding the fundamental role of calcium in orchestrating the transduction of the gravity stimulus into an autopoietic (self-regulated) response.

The project brings together experts in a range of specially-selected fields to address a single major research problem, i.e., the fundamental role of calcium in regulating gravity-stimulated signal transduction in plants. The expertise to be called on includes molecular biologists to produce transgenic plants with altered calcium homeostasis (Thompson, Robertson, Sederoff); cell biologists to image calcium and other components of the

signal transduction pathway (Allen, Tucker); physiologists to study signal transduction (Boss, Davies, Muday); and biochemists (Brown, Huber, Huber) to study calcium-modulated carbon/nitrogen metabolism. By fostering interdisciplinary collaborations among these diverse laboratories, the proposed program will create a multi-faceted approach to the study of plant gravitational biology.

The NSCORT is a consortium of institutions including North Carolina State University (College of Agriculture and Life Sciences and School of Forestry), Wake Forest University, Baruch College (City University of New York), NASA's Kennedy Space Center and Dynamac Corporation (which runs the Life Science Support contract at KSC). Faculty, staff and/or students from all of the institutions participate in various aspects of the program. It consists of three major components: Education, Outreach, and Research. Considerable progress has been made in all three areas.

Education: The NSCORT program offered the graduate-level course "Gravitational and Space Biology" to 15 graduate and advanced undergraduate students (Fall 1996). This course originated from the NC State University campus and was available on a real-time basis at 5 other campus locations throughout the state via the NC Regional Electronics Network microwave system. A new undergraduate level course "Space Biology" was initiated and taught to 16 students (Fall 1997) at the NC State University campus. A portion of this course was taught remotely from the Kennedy Space Center using real time audio and video link-ups. Experts in space biology (including Astronaut/Physician Dr. Chuck Brady) were brought in to expose students to current activities and opportunities in the field.

Outreach: A summer workshop for high school teachers entitled "Plants and Gravity" was offered. Ten teachers from five states spent time at the NC State University campus interacting with project leaders and research associates in the program. Some of the teachers have utilized the experience to develop new curricula at their schools. Additionally, a two day workshop was sponsored and held at the NC State University campus to train teachers for the educational opportunities associated with the Collaborative Ukrainian Experiment. As a group, the NSCORT participated in two separate science and technology symposia that offered middle school students, particularly girls, an opportunity to conduct hands-on experiments in a wide range of scientific areas. The NSCORT offered activities and tours at the North Carolina Science Olympiad and the National Science Olympiad. NSCORT scientists presented demonstrations and activities during science fairs at two local elementary schools and hosted a workshop on plant gravitational and space biology for middle and high school students attending the Imagination Station Summer Science Camp.

Research: In order to take an integrated approach to studying graviperception and response, six postdoctoral fellows were hired. These individuals possess skills in molecular biology, electrophysiology, biochemistry, and cell biology. Four graduate students are currently in the program and one or two more will be recruited. The physical set-up of the laboratories is complete, including the renovation of the electrophysiology laboratory and the acquisition of a Leica DMRXA confocal microscope with a rotatable stage. This piece of equipment will allow us to examine the real time *in vivo* imaging response of graviresponding plant tissue.

Focus has been placed on the maize pulvinus and the pine seedling compression wood systems as models to dissect biochemical and morphological response to gravity. Initial characterization is complete and detailed studies are underway. To understand the molecular genetic component of the gravity response in plants, we have chosen three proteins (calreticulin, calmodulin, and phosphatidylinositol kinase) to clone. These clones will then be used to transform *Arabidopsis* plants for studies on the influence of altered calcium homeostasis in the gravity response.

This research will determine the mechanisms by which plants perceive and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions will improve our understanding of how they grow in various space conditions (Earth orbit, Mars) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products of enhanced paper quality (pine seedling system and its formation of compression wood) and yield of seed grains (reorientation of corn plants blown over in strong winds).

FY97 Publications, Presentations, and Other Accomplishments:

- Allen, N.S. "Confocal microscopy and GFP imaging." (lecture) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "Darkfield and phase microscopy, the basics." (lecture) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "Deconvolution, what is it and where should you use it." (lecture) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "From Leeuwenhoek to electronic light microscopy." (keynote address) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "Resolution. Abbe's diffraction experiments." (lecture) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "Polarization and DIC microscopy, theory and practice." (lecture) Dept. of Medical Microbiology, Linköping University, Linköping, Sweden. Video Microscopy Short Course (August 25 - September 4, 1997).
- Allen, N.S. "Tip growth, an overview." Cytonet Conference, Breckenridge, CO (1997).
- Allen, N.S. and Bennett, M.N. "Electro-optical imaging of F-actin and endoplasmic reticulum in living and fixed plant cells" in "Science of Specimen Preparation for Microscopy and Microanalysis." Edited by: Malecki, M. and Roomans, G. SMI Press, AMF O'Hare, Chicago, IL (In Press).
- Allona, I., Quinn, M., Sun, Y-H., Shoop, E., Swope, K., St. Syr, S., Retzel, E., and Whetter, R. "Analysis of gene expression during xylem differentiation in loblolly pine." (Abstract) *Plant Physiol.*, 114(3), 11 (1997).
- Boss, W.F., Perera, I.Y., Ransom, W.D., Stevenson, J.M., Brglez, I., Heilmann, I.H., and Gross, W. "Wrapping about the cytoskeleton." (Abstract) *Plant Physiol.*, 114(3), 15 (1997).
- Brady, S.R., Dixon, M.W., Cyr, R.J., Fisher, D.D., and Muday, G.K. "BY2 protoplasts as a model for NPA sensitive auxin transport and gravitropism." (Abstract) *ASGSB Bull.*, 10(1), 78 (1996).
- Brown, C.S. "Plant growth and physiology in space." Baruch College, Manhattan, NY (March 4, 1997).
- Brown, C.S. "Plant metabolism in space." University of North Carolina-Pembroke, Pembroke, NC (March 13, 1997).
- Brown, C.S. "The Collaborative Ukrainian Experiment: Scientific overview." Presented to the 12th Man In Space Symposium, Washington DC (June 13, 1997).
- Brown, C.S. (co-authors - Sanwo, M.M., Stryjewski, E.C., Peterson, B.V., Johnson, C.F., Piastuch, W.C., Hilaire, E., and Guikema, J.A.) "The interaction of microgravity and ethylene on soybean growth and metabolism." Presented to the 12th Man In Space Symposium, Washington DC (June 11, 1997).
- Brown, C.S., Sanwo, M.M., Hilaire, E., Guikema, J.A., Stryjewski, E.C., and Piastuch, W.C. "Starch metabolism and ethylene production in space-grown soybean seedlings." (Abstract) *ASGSB Bull.*, 10, 34 (1996).

- Brown, C.S., Sanwo, M.M., Stryjewski, E.C., Peterson, B.V., Johnson, C.F., Piastuch, W.C., Hilaire, E., and Guikema, J.A. "Carbohydrate metabolism and growth in space-grown soybean." (Abstract) *Plant Physiol.*, 114(3), 91 (1997).
- Brown, D. and Muday, G. "Testing the hypothesis that flavonoids regulate auxin transport *in vivo*." (Abstract) 8th International Conference on *Arabidopsis* Research. 4-8. (1997).
- Brown, D.E., Reed, R.C., and Muday, G.K. "Polar auxin transport and gravitropism in *Arabidopsis thaliana*." (Abstract) *ASGSB Bull.*, 10(1), 38 (1996).
- Butler, J.A., Hu, S., Brady, S., Dixon, M.W., and Muday, G.K. "*In vitro* and *in vivo* evidence for actin association of the NPA binding protein." *Plant J.* (In Press).
- Carter, C.N. and Allen, N.S. "Localization of putative L-type calcium channels in living higher plant cells using the fluorescent dye DM-BODIPY DHP." (Abstract) *Plant Physiol.*, 114(3), 275 (1997).
- Collings, D. (co-authors - Winter, H. and Allen, N.S.) "Cytoskeletal organization in gravistimulated maize stems." *New England Society for Microscopy*. Woods Hole, MA (May, 1997).
- Collings, D., Winter, H., Huber, J.L., and Allen, N.S. "Structural analysis of gravistimulated maize stems." (Abstract) *Plant Physiol.*, 114(3), 86 (1997).
- Croxdale, J.G., Cook, M.E., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. "Structural aspects of potato tubers formed in space." (Abstract) *ASGSB Bull.*, 10(1), 13 (1996).
- Croxdale, J.G., Cook, M., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. "Structure of potato tubers formed in microgravity." *J. Exp. Bot.* (In Press).
- Davies, E. (invited seminar) Department of Botany, University of Washington, Seattle (1997).
- Davies, E. (invited speaker) University of California-Riverside Symposium on Post-transcriptional Regulation of Gene Expression (1997).
- Davies, E. (plenary speaker) *Molecular Biology of Plants under Environmental Stress*, Poznan, Poland (1997).
- Heilmann, I., Gross, W., and Boss, W.F. "Polyphosphoinositide metabolism in *Galdieria sulphuraria*." (Abstract) *Plant Physiol.*, 114(3), 268 (1997).
- Holzwarth, G., Webb, S.J., Kubinski, D.J., and Allen, N.S. "Improving DIC microscopy with polarization modulation." *J. Microscopy* (In Press).
- Holzwarth, G., Webb, S.J., Kubinski, D.J., and Allen, N.S. "Improving DIC microscopy with polarization modulation." (Abstract) *Mol. Biol. Cell*, 7, 158A (1996).
- Hu, S., Dixon, M.L., and Muday, G.K. "Development of a procedure to depolymerize the actin cytoskeleton for purification of associated proteins." (Abstract) *ASGSB Bull.*, 10(1), 27 (1996).
- Huber, J.L., Winter, H. and Huber, S.C. "Do Ca⁺⁺ and protein phosphorylation play a role in the gravitropic response?" (Abstract) *Plant Physiol.*, 114(3), 272 (1997).
- Johnson, K.M., Brown, C.S., and Piastuch, W.C. "Examination of the expression patterns of genes involved in carbon metabolism in soybean seedlings grown in space." (Abstract) *Plant Physiol.*, 114(3), 253 (1997).

- Luciano, R.L. and Muday, G.K. "Characterization of gravity dependent growth in *Fucus zygotes*." (Abstract) ASGSB Bull., 10(1), 26 (1996).
- MacKay, J., O'Malley, D.M., Presnell, T., Booker, F.L., Campbell, M.M., Whetten, R.W., and Sederoff, R.R. "Inheritance, gene expression, and lignin characterization in a mutant pine deficient in cinnamyl alcohol dehydrogenase." PNAS, 94, 8255-8260 (1997).
- Patent Approved, U. S. Patent #: Undetermined. MacKay, O'Malley, Whetten, and Sederoff. "Method of altering lignin in trees."
- Muday, G.K. "The role of auxin transport and the actin cytoskeleton in plant gravity response." Joint NSF/NASA NSCORT Meeting, Kennedy Space Center, FL (February 17, 1997).
- Muday, G.K. and Reed, R.C. "Inhibition of auxin movement from the shoot into the root inhibits lateral root development in wild-type *Arabidopsis thaliana* and *alf1-1*." (Abstract) 8th Intl. Conf. on *Arabidopsis* Res. 4:41 (1997).
- Muday, G.K., Butler, J.H., Hu, S., Brady, S., and Dixon, M.W. "Actin association of the naphthylphthalamic acid binding protein from zucchini hypocotyls." (Abstract) ASGSB Bull., 10(1), 55 (1996).
- O'Malley, D.M., MacKay, J.J., Whetten, R., Campbell, M., Liu, B-H., and Sederoff, R.R. "Genomic approaches to manipulating lignin composition and amount in wood." TAPPI Proceedings pp.105-109 (1996).
- Perera, I.Y., Stevenson, J.M., and Boss, W.F. "Investigation of phosphoinositide metabolism in gravistimulated maize pulvini." (Abstract) Plant Physiol., 114(3), 284 (1997).
- Ralph, J., MacKay, J., Hatfield, R., Whetten, R.W., O'Malley, D.M., and Sederoff, R.R. "Abnormal lignin in a loblolly pine mutant." Science, 277, 235-239 (1997).
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- Scott, A.C. and Allen, N.S. "The effect of carbonylcyanide m-chlorophenylhydrazone (CCCP) application on the gravitropic response in roots and stamen hairs." (Abstract) ASGSB Bull., 10(1), 17 (1997).
- Sederoff, R.R. Sigma Xi invited lecture, BASF, Research Triangle Park, NC (1997).
- Sederoff, R.R. Workshop presentation, Agricultural Biotechnology, Uppsala, Sweden (1997).
- Sederoff, R.R. (invited seminars) Horticultural Research, Auckland, New Zealand (1996); Forestry Research Institute, Rotorua, New Zealand (1996); Department of Chemistry, University of Ohio (1997); ForBio Research Ltd. Brisbane (1997); University of Arizona, Tucson (1997); University of Stellenbosch, South Africa (1997).
- Sederoff, R.R. (invited speaker) Forest Tree Workshop, Plant & Animal Genome 5 (1996); University of Chicago, Genetics Minisymposium (1997); Institute of Paper Science and Technology, Atlanta, GA (1997); Chinese Academy of Forestry, Beijing, China (1997); Nanjing Forestry University, Nanjing, China (1997); Presidents Circle, National Academy, Woods Hole, MA (1997); Molecular Genetics of Forest Trees, IUFRO, Quebec (1997); Internat. Soc. Plant Molecular Biology, Congress in Singapore (1997).
- Sederoff, R.R. (symposium speaker) Swedish Plant Physiology Society, Uppsala (1997); Amer. Assoc. Plant Physiology, Vancouver (1997); Eucalypt: IUFRO meeting, Salvador, Brazil (1997); Forest Biotechnology Conf., Blomfontein, South Africa (1997).

Tibbitts, T.W., Croxdale, J.G., Brown, C.S., and Wheeler, R.M. "Potato tuber growth and starch accumulation in space." (Abstract) ASGSB Bull., 10(1), 28 (1996).

Vian, A., Henry-Vian, C., and Davies, E. "Molecular analysis in tomato of stree related genes in relation to electrical signal." (Abstract) Plant Physiol., 114(3), 278 (1997).

Winter, H., Huber, J.L., and Huber, S.C. "Sucrose metabolism during gravity induced cell elongation." (Abstract) Plant Physiol., 114(3), 143 (1997).

Winter, H., Huber, J.L., Collings, D., Allen, N.S., and Huber, S.C. "Carbohydrate metabolism in graviresponding maize pulvini." (Abstract) ASGSB Bull., 10(1):extra (1996).

Wyatt, S.E. "Gravity, calcium, signalling & *Arabidopsis*." Plant Physiology Seminar, Dept. of Botany, Duke University, Durham, NC (March, 1997).

Wyatt, S.E., Brown, D., Robertson, D., and Muday, G. "The gravitropic response of the inflorescence stems of *Arabidopsis*." (Abstract) Plant Physiol., 114(3), 133 (1997).

Wyatt, S.E., Tsou, P-L., and Robertson, D. "Manipulation of calcium homeostasis with calreticulin." (Abstract) Plant Physiol., 114(3), 274 (1997).

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 Edgar Spalding; University of Wisconsin
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NOTE: This NSCORT represents nine individual tasks with nine principal investigators.

Funding:

UPN/Project Identification: 199-93-17-11

Solicitation: not available

Initial Funding Date: 1994

Expiration: 1999

Students Funded Under Research: 32

Post-Doctoral Associates: 13

FY 1997 Funding: not available

Joint Agency Participation: National Science Foundation

Task Description:

This joint program supports a network of researchers with complementary skills and ideas who focus on the study of how plants sense and respond to various environmental signals, such as light, gravity, and mechanical perturbations. One of the major goals of the program's collaborative research network is to elucidate pathways of signal transduction in plant sensing and determine the manner in which they are connected to the growth and physiological responses that allow plants to adapt or adjust to varying environmental conditions.

During FY97, much of the research effort of the network was focused on the super project decided upon at a research planning meeting held in Half Moon Bay, California in the spring of 1996. The super project focuses on the physiological and molecular characterization of the distal elongation zone in roots as a central project on plant sensory perception. Our annual research progress meeting for 1997 was held at Timberline Lodge, Oregon on September 4 - 7, 1997. During this meeting, reports on progress on the super project as well as on individual PI projects were presented.

The key goals for the first year of the super project included: (1) to isolate sufficient quantities of root tissue subsections from *Arabidopsis* to allow sufficient extraction of mRNAs to do differential display analysis of gene activation in the tissue; (2) continue the technical advances in the gene microarray system to allow determination of genes uniquely expressed in the DEZ; (3) characterize the electrophysiological properties of cells from the DEZ; and (4) characterize the physiological properties of cells of the DEZ and gain additional information on the localization of the DEZ relative to the meristem and central elongation zone. Advances in these areas during the

past year have included: (1) the successful isolation of large numbers of specific tissue samples from the specific zones of the root, extraction of mRNAs from these tissue regions and use of the samples for differential display analysis of gene expression along the root apex; (2) continued improvements in the Davis laboratory on the automated gene microarray assay technology needed for analysis of the isolated mRNAs; (3) isolation of protoplasts from the DEZ of *Arabidopsis* roots and analysis of their ion channel activity tissue; and (4) detailed computer video analysis of the distribution of surface extension patterns along the apex of the *Arabidopsis* root including analysis of the interaction of specific tissue regions during expression of the gravitropic response. These experiments have been coupled with analysis of the effects of anion channel inhibitors on growth and gravitropism as experiments complementary to the above-mentioned analysis of ion channel activity in protoplasts from the DEZ. Key results from these experiments include the finding that tissue samples on the order of 1000 units from each region are enough to allow extraction of quantities of mRNA sufficient for differential display and for gene microarray analysis (although a limited number in the latter case). The initial differential display analyses are somewhat disappointing in that relatively few genes seem to be specifically expressed within the DEZ. We are examining whether there is a lack of one-to-one correlation between the anatomical appearance of the DEZ and the chronology of gene expression patterns. Also, as our computer analysis of surface expansion patterns in the *Arabidopsis* root tip becomes more sophisticated, we may be able to pin down the DEZ with more precision. The analysis of channel activity in DEZ protoplasts reveals a highly active anion channel (Cl⁻) that is voltage dependent and inhibited by the anion channel blocker, nitrophenylpropylbenzoic acid (NPPB). Interestingly, this anion channel blocker eliminates the gravitropic response of the DEZ with little or no effect on root growth, suggesting that channel activation may be a key component of the gravitropic signaling pathway. Recent studies indicating that the NPPB effect may be quite specifically related to the gravitropic response (in addition to the fact that it has little effect on growth per se) include experiments indicating that NPPB is effective only when applied to the upper side of the DEZ (the region that exhibits strong stimulation-induced growth enhancement during the gravitropic response). In addition to these experiments, we have made progress in characterizing the role of the DEZ in other root responses, especially responses to touch and expression of root. Progress on individual PI projects has also continued along with collaborative efforts among network researchers (see publications and presentations).

The research in each network laboratory focuses on specific aspects of signal transduction related to plant responses to the environment. The projects include molecular and physiological analyses of plant responses to gravity, touch, light, and hormones and in most cases, the emphasis is on subcellular mechanisms that mediate such plant responses. Knowledge gained from this research should significantly improve our understanding of how plants interact with important environmental signals. As we gain more information on mechanisms of plant responses to environmental challenges, we will improve our ability to optimize plant growth under a variety of conditions including optimization of plant performance under less than ideal conditions on Earth as well as optimization of growth in unique environments such as those encountered during space flight.

In addition to these benefits, there are two general benefits to the research community to be realized from network activity. One derives from our progress in cloning genes from each developmental region of the *Arabidopsis* root. Once cloned, these genes will be useful tools for the research community at large in a wide variety of investigations of plant development and sensing. A second benefit derives from the network's ongoing development of a plant growth imaging web site devoted to automated analysis of plant growth and standardization of plant growth experimental conditions. This is anticipated to provide a means for greatly accelerating our progress in the understanding of plant growth.

FY97 Publications, Presentations, and Other Accomplishments:

Armstrong, F., Ptak, C., and Assmann, S.M. "Complexities of the maize stomatal complex." (Poster) ASPP/CSPP annual meeting, Vancouver, B.C. *Plant Physiol.* 114: 197 (1997).

Assmann, S.M. "K⁺ currents in the maize stomatal complex." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997).

Assmann, S.M. and Haubrick, L.L. "Transport proteins of the plant plasma membrane." *Curr. Op. Cell Biol.*, 8, 458-467 (1996).

- Assmann, S.M. and Romano, L.A. "Secondary messenger regulation of ion channels/Plant patch clamping" in "Methods in Enzymology." Edited by: Conn, R. Academic Press: San Diego, CA (In Press).
- Assmann, S.M., Ptak, C., and Armstrong, F. "Potassium channels in the maize stomatal complex." U.S. Crop Science/Agronomy Meeting, Indianapolis, IN (1996).
- Brandstatter, I. and Kieber, J. "Identification of cytokinin primary response genes from *Arabidopsis thaliana*." (Poster) 7th International *Arabidopsis* Meeting, Madison, WI (1997).
- Davis, R. "Whole genome analysis of *Saccharomyces cerevisiae*." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997).
- DeLong, A. "Using dominant defective mutants to analyze PP2A function in *Arabidopsis*." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997).
- DeLong, A. "Using dominant defective mutants to analyze protein phosphatase 2A function *in vivo*." Presented at the annual retreat of the Department of Molecular Biology, Cell Biology and Biochemistry, Brown University (1997).
- DeLong, A., Deruere, J., and Söll, D. "Functions of an *Arabidopsis* protein phosphatase 2A subunit." (Poster) Presented at the annual retreat of the Department of Molecular Biology, Cell Biology and Biochemistry, Brown University (1997); "Functions of an *Arabidopsis* protein phosphatase 2A subunit." (Poster) 8th International Conference on *Arabidopsis* Research, Madison, WI (June 25 - 29, 1997).
- Evans, M. "Molecular and physiological characterization of the distal elongation zone in roots." Joint Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997); "The NASA/NSF Joint Program in Plant Biology. Activities of the Network for Research on Plant Sensory Systems." Joint Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997); "Thigmotropism, gravitropism and circumnutation in *Arabidopsis* roots: High resolution analysis of the role of the distal elongation zone." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997).
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- Harper, J. "Pumps and kinases: Are they at the root of our problem?" Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997); "Calcium dependent protein kinases and calcium pumps." Joint NSCORT meeting, Kennedy Space Center (February, 1997); "Coding and decoding calcium signals in plant." Copenhagen Biological Society (April, 1997); "Regulation of a CDPK by intra-molecular binding of the calmodulin-like regulatory domain." Plant Kinase Meeting, Jackson Hole, WY (1997).
- Harper, J.F., Hong, B., Hwang, I., Guo H.Q., Stoddard, R., Huang, J.F., Palmgren, M.G., and Sze, H. "A novel calmodulin-regulated Ca^{2+} -ATPase (ACA2) from *Arabidopsis* with an N-terminal autoinhibitory domain." *J. Biol. Chem.* (In Press).
- Henriksen, G.H. and Assmann, S.M. "Laser-assisted patch clamping: A methodology." *Pfluegers Archiv/ Eur. J. Physiol.*, 433, 832-841 (1997).

- Huang, J.F. Intramolecular activation of a calcium dependent protein kinase. (Dissertation) The Scripps Research Institute (1997).
- Huang, Y., Li, H., and Kieber, J. "Biochemical and molecular characterization of CTR1, a protein kinase involved in ethylene signaling." (Poster) 7th International Arabidopsis Meeting, Madison, WI (1997).
- Ishikawa, H. and Evans, M.L. "Novel software for analysis of root gravitropism: Comparative response patterns of *Arabidopsis* wild type and *axr1* seedlings." *Plant, Cell & Environ.*, 20, 919-928 (1997).
- Ishikawa, H. and Evans, M.L. "Use of computerized video imaging technology for analysis of differential growth patterns during root gravitropism." First Annual Meeting, NASA Specialized Centers of Research and Training, Kennedy Space Center, FL (February 12 - 14, 1997).
- Johnson, K., Praetorius-Ibba, M., and Söll, D. "Searching for components of the protein phosphatase 2A signalling pathway." (Poster) 7th International Arabidopsis Meeting, Madison, WI (1997).
- Karlin-Neumann, G. "Status of the gene microarray technology." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997); "Touch-sensing in *Arabidopsis*: From mutants to gene microarrays." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997).
- Karlin-Neumann, G. and Davis, R.L. "Mechanoluminescence mutants of *Arabidopsis*." Stanford Biochemistry Department Annual Retreat (1996); "Use of engineered mechanoluminescence to analyze mechanosensing in *Arabidopsis* mechanoluminescence mutants of *Arabidopsis*." Stanford Plant Biology Annual Retreat (1996).
- Kieber, J. "Molecular genetic analysis of ethylene and cytokinin signaling in *Arabidopsis*." (1997).
- Kieber, J. "Progress on mRNA isolation from *Arabidopsis* DEZs." Annual Meeting, NASA/NSF Joint Program in Plant Biology, Timberline Lodge, OR (1997); "Genetic and molecular analysis of ethylene and cytokinin signaling in *Arabidopsis*." Indiana University, Bloomington (1997); "Genetic and molecular analysis of ethylene and cytokinin signaling in *Arabidopsis*." Chicago Area Plant Biology Meeting, University of Illinois at Chicago (1996).
- Kieber, J.J. "The ethylene response pathway in *Arabidopsis*." *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48, 277-296 (1997).
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Task Description:

Research at NJ-NSCORT is performed through a series of four separate, interacting research teams. The tasks listed below in many cases depend on or feed into research performed by other teams.

Biomass Production Team

- 1) Develop a Plant Production Decision Support System Assisted by Machine Vision Monitoring of Plant Stress.
 - Identify for feature extraction spectral quality change and quantify morphological change due to temperature-induced stress of tomato plants.
 - Develop an integrated sensing system for plant development monitoring.
 - Establish automated control algorithms correcting the effects of the variances for management of a biomass production sub-system (PBS) within a bioregenerative life support system (BLSS).
- 2) Investigate Environmental Control of Tomato Production: Temperature Effects on Growth, Yield and Fruit

Quality.

- Investigate the effects of perturbations in air temperature on tomato growth, physiology, and yield.
 - Correlate fruit quality parameters with temperature changes and whole plant growth and developmental state.
 - Establish a relationship between altered sink strength and AGP gene(s) expression.
- 3) Modeling and Optimization of Biomass Production Systems Including a Hypermedia Application.
- Collect data and existing models as inputs to the sub-system modeling process.
 - Develop an application that will serve as both a decision-making tool and a computer-based testing vehicle that will facilitate training.

Food Processing and Nutrition Team

- 1) Plan and coordinate a conference to define research needs for nutritional issues related to long-term space flight. The information generated by this conference will be used to further define the nutritional needs of space voyagers in stressed, low-gravity conditions, to develop palatable menus for space voyagers, and to recommend plants for a BLSS to ensure a complete supply of all essential nutrients.
- 2) Use the crops that have been selected by ALS investigators to produce a palatable and nutritious menu for long-term space flight. Studies will begin with soy beans and wheat to produce a variety of ingredients.
- 3) Develop mathematical simulation necessary to develop a versatile and miniaturized extruder suitable for a life support environment.

Waste Processing and Resource Recovery

- 1) Volatile Organic Air Contaminants: Identification, Monitoring and Control.
- Identify and quantify amounts of volatile air contaminants that will enter a BLSS atmosphere from: humans, plants, food processing, and waste treatment and from a database of these contaminants.
 - Develop regenerative treatment methods for removing those volatile contaminants from the BLSS atmosphere.
 - Develop a continuous monitoring system that can be used to control operation of the regenerative treatment system and thus ensure the maintenance of satisfactory air quality on the BLSS.
- 2) Recovery of Nutrients from Non-Edible Plant Material.
- Characterize the non-edible portion of tomato plants.
 - Compare the results of heat treatment and nutrient addition on the rate and efficiency of the degradation process.
 - Construct and operate a bench-scale anaerobic/aerobic treatment system.
- 3) Non Linear Empirical Modeling and Optimization of Waste Recovery Systems.
- Machine readable input-output data for a variety of waste treatment processes.
 - Provide input for "Global Optimization" project to Systems Studies and Modeling team.

Systems Studies and Modeling Team

- 1) Automation-Culture-Environment Oriented Analysis for Bioregenerative Life Support Systems.
- Establish an NJ-NSCORT computer network to facilitate operations analysis capability to support planning, analysis, requirements definition, design, and possibly control of a BLSS.
 - Perform a systems abstraction and formulate description of operation schemes within a BLSS
 - Develop system requirements and database and software framework for ACE_SYS.
- 2) Global Optimization of a Complex Integrated Systems for Bioregenerative Life Support.
- Review existing models related to systems integration, analysis and information flow within a BLSS.
 - Define key variables that interact with other modules in a BLSS.

- Collect data and models from principal investigators and research associates in each project and generate polynomial submodels based upon that information.

FY97 Progress:

Biomass Production Team

1) Develop a Plant Production Decision Support System Assisted by Machine Vision Monitoring of Plant Stress.

- Identification of tomato plant feature extraction was performed for spectral quality change and quantified morphological change due to temperature-induced stress of tomato plants.

2) Investigate Environmental Control of Tomato Production: Temperature Effects on Growth, Yield, and Fruit Quality.

- The effects of perturbations in air temperature on tomato growth and physiology from germination to first flower were investigated and correlated to machine vision images.

- An established relationship between altered sink strength and AGP gene(s) expression has begun.

3) Modeling and Optimization of Biomass Production Systems Including a Hypermedia Application.

- Data and existing models were collected for inputs to the sub-system modeling process.

- An application was developed that will serve as both a decision-making tool and a computer-based testing vehicle that will facilitate training. Development continues.

Food Processing and Nutrition Team

1) Development has started and will continue for mathematical simulation necessary to develop a versatile and miniaturized extruder suitable for a life support environment.

Waste Processing and Resource Recovery

1) Volatile Organic Air Contaminants: Identification, Monitoring and Control.

- Identification and quantification amounts of volatile air contaminants that will enter a BLSS atmosphere from: humans, plants, food processing and waste treatment has begun and the database of these contaminants is in further development.

2) Biofilters have been developed for regenerative treatment for removing volatile contaminants from the BLSS atmosphere.

3) Recovery of Nutrients from Non-Edible Plant Material.

- The non-edible portion of the tomato plant has been characterized.

- Have begun to compare the results of heat treatment and nutrient addition on the rate and efficiency of the degradation process.

- Have constructed and begun to operate a bench-scale anaerobic/aerobic treatment system.

4) Non Linear Empirical Modeling and Optimization of Waste Recovery Systems.

- Have provided input for "Global Optimization" project to Systems Studies and Modeling team.

Systems Studies and Modeling Team

1) Automation-Culture-Environment Oriented Analysis for Bioregenerative Life Support Systems.

- Established a NJ-NSCORT computer network to facilitate operations analysis capability to support planning, analysis, requirements definition, design, and possibly control of a BLSS.

- Have begun to perform a systems abstraction and formulate description of operation schemes within a BLSS

- Completing development of system requirements and database and software framework for ACE_SYS.
- 2) Global Optimization of a Complex Integrated Systems for Bioregenerative Life Support.
- Reviewed existing models related to systems integration, analysis and information flow within a BLSS.
 - Defined key variables that interact with other modules in a BLSS.
 - Continually collecting data and models from principal investigators and research associates in each project and generating polynomial submodels based upon that information.

NSCORT research on agricultural efficiency, food processing, and waste management will help solve problems we face today in our farms, factories, and backyards. NSCORT research is particularly valuable in urbanizing areas, which must solve problems of declining farmland, waste management, and agricultural profitability.

Limited resources and agricultural productivity

NJ-NSCORT is developing methods to maximize the production of edible crops in an enclosed area while at the same time conserving and recycling as much of the water and nutrients as possible. The machine vision system developed by NJ-NSCORT's biomass production team offers a way to continuously monitor crop performance. We also are generating the data necessary to develop a crop growth model that predicts the effects of temperature changes on harvest date.

Our food processing team is developing a model to better predict how small-scale food extruders will work. Extrusion is a versatile food processing system that simultaneously mixes, cooks, and shapes food. The end product of this work could be a home-scale food extruder that would allow families to create a wide variety of foods from customized breakfast cereals to breads and chips.

Environmental Management

Our waste management team designed a new apparatus to sample the headspace around plants for trapping and measuring volatile compounds. A second group within our waste management team has designed a biofilter to remove ammonia from air and design of a biofilter for ethylene removal has begun. A third group within the waste management team has conducted preliminary experiments to maximize recovery of nutrients from the non-edible portion of plants, which will help increase the attractiveness of recycling of this portion of the organic waste stream.

FY97 Publications, Presentations, and Other Accomplishments:

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Sauser, B.J. "The use of a logistic plant growth model for prediction of tomato plant development based on air temperature." Presentation for the Department of Bioresource Engineering. New Brunswick, NJ. (October, 1996).

Sauser, B.J. "What temperature do you really want? Getting the most out of a growth chamber." Presentation for the Association of Education and Research Greenhouse Curators 1997 Annual Meeting. New Brunswick, NJ (July, 1997).

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Ting, K.C., Ling, P.P., and Giacomelli, G.A. "Sustaining human lives in outer space." *Resource* (March 7 - 8, 1997).

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NSCORT: Gravitational Biology

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NOTE: This NSCORT represents nine individual tasks with nine principal investigators.

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Post-Doctoral Associates: 7

Task Description:

The Rice NSCORT research program is focused on examining the effects of microgravity and associated stresses on development and cell culture in prokaryotes and eukaryotes. While most of the research involves mammalian cells, research is also underway on plant cells, yeasts, microbial cells, and *Drosophila melanogaster*. Many of the projects include utilization of the rotating bioreactor systems developed at Johnson Space Center as a tool for simulation of some aspects of the microgravity environment. The model systems have been carefully chosen so that specific mechanistic questions at the cellular and molecular level can be addressed and so that once the proper ground-based data have been assembled, the development of flight-based proposals will not be difficult.

PROJECT I: THE ROLE OF GRAVITY IN EARLY EMBRYONIC PATTERN FORMATION. One of the well-characterized behavioral responses of *Drosophila* involves sensing the vector of the gravitational force. Thus, when adult *Drosophila* are tapped to the bottom of a glass vial, they respond by quickly climbing up the vial walls in the opposite direction to the gravitational vector (the "climb" test). This response occurs in the absence of light and other stimuli, indicating that it is purely a response to the direction of the gravitational force. We are using this assay in combination with random mutagenesis of the organism (by chemical mutagens) to isolate mutants defective in this test. This will ultimately lead to identification of the genes that mediate the gravitational response. Secondary testing of all mutants identified in the first screen is being performed to eliminate individuals with simple motor defects that interfere with their ability to respond to gravity. The small size of *Drosophila*, the simplicity of the screening test, and the use of a so-called F1 screening protocol are allowing us to screen very large numbers of potential mutants. To date, ~10,000 flies

have been examined and at least one potential mutation has been identified. Data from previous space flights indicates that hypogravity has an effect upon the early events of embryogenesis in *Drosophila*. Given that many of the effects of hypogravity reflect perception of altered mechanical forces, our working hypothesis is that the embryonic defects produced in *Drosophila* by hypogravity may involve altered Ca^{2+} movement and mobilization in the early embryo. We have therefore initiated experiments to visualize Ca^{2+} localization and movement in the early embryo both under normal and altered gravitational forces.

PROJECT II: THE ROLE OF GRAVITY IN THE DEVELOPMENT AND FUNCTION OF THE *DROSOPHILA* NERVOUS SYSTEM. The following specific aims were proposed for this project: a) effects of gravity on establishment of neuronal identity; b) effects of gravity on axon pathfinding; and c) effects of gravity on synaptic function. Several previous reports suggested that exposure of a developing organism to altered gravity, either microgravity or hypergravity, could perturb development or function of the nervous system in subtle but specific ways. These perturbations caused changes in the behavior of the organism at the adult stage, which were observed even after return to normal gravity. The behavioral changes observed were most consistent with abnormalities in the vestibular system, which enables the organism to orient itself properly in space. These effects of altered gravity on the nervous system might be important factors in considering space flights of long duration. Thus it is of interest to define these effects in more detail, and to try to elucidate the cellular and molecular mechanisms by which these effects are generated. Preliminary Data Obtained: Structure of the central and peripheral nervous system in late stage embryos was determined following two spinning protocols: first, embryos that were spun from 3-6 hours post fertilization, and then those that were spun from 2-17 hours post fertilization. Following these spins, embryos were stained with monoclonal antibody 22C10, which recognizes the cell bodies, axons and dendrites of all neurons, and then dissected to enable visualization. Both the CNS and PNS look normal at this level of analysis. If we can confirm that our various spinning protocols have no observable effect on the structure of the CNS or PNS as assayed by antibody 22C10, we will use other available antibodies that enable the visualization of subclasses of neurons and axons which will allow us to test for more subtle effects of altered gravity on the nervous system.

PROJECT III: MOLECULAR AND DEVELOPMENTAL RESPONSES OF PLANTS TO MECHANICAL STIMULI. The major focus of this work is to determine the *in vitro* and *in vivo* functions of the calmodulin-related *TCH2* gene product of *Arabidopsis thaliana*. Expression of *TCH2* is strongly and rapidly upregulated by stimuli such as wind, temperature, stress, and darkness; thus, *TCH2* may function in the adaptation of plants to environmental stresses. The specific aims of this work are as follows: 1) Tissue and subcellular localization of *TCH2*; 2) *In vitro* activity of *TCH2*; and 3) Physiological functions of *TCH2*. Results and Plans for the Coming Year: To probe the localization of *TCH2* protein in plants, we have generated *TCH2*-specific antibodies. One lot of antibodies shows a specific interaction with a single band of the appropriate size of *TCH2* on Western blots of total plant protein. This band is most likely *TCH2* because the protein shifts size in the presence of Ca^{2+} (a characteristic of calmodulin-like proteins) and because the antibody does not recognize purified calmodulin. The antigenic band also increases in abundance in plants exposed to darkness, a stimulus known to increase *TCH2* transcript levels. Thus, we are now poised to begin Western analyses of the accumulation of *TCH2* protein during development and in plants exposed to various environmental stimuli. These antibodies will enable us to determine the cell and tissue localization of *TCH2* protein using light microscopy and to identify the subcellular localization using immuno-electron microscopy. The second specific aim is to characterize the *in vitro* activity of *TCH2*. Currently, we are attempting to produce in *E. coli* "native" *TCH2* protein (i.e., lacking any sequence modifications from *TCH2* protein produced in plant cells). This protein will be purified by phenylsepharose and then will be used in assays to determine if *TCH2* can modulate the activity of known calmodulin targets. The analyses described above lead into our overall goal of this work which is to determine the cellular functions of *TCH2*. One direct approach is to identify plants that are altered in their expression of *TCH2*. We have discovered through related work in the laboratory that *TCH2* expression may be turned on strongly in guard cells during the process of stomatal closure. This response is known to be influenced by cytosolic Ca^{2+} levels and by the ABI-1 protein phosphatase. It is possible, therefore, that *TCH2* may function in the process of turgor loss in guard cells. To test this possibility, we will determine (i) whether *TCH2* can directly bind ABI-1 using the gel overlay assays described above and the yeast two-hybrid screen and (ii) whether *TCH2* can influence guard cell ion channel

activity in collaboration with Julian Schroeder's laboratory. We will provide them with antibodies and TCH2 protein to determine if the loss or gain of TCH2 availability in guard cells affects channel activity. In addition, when antisense and sense plants have been sufficiently characterized, the Schroeder laboratory will determine if channel activity in the guard cells from these plants are significantly affected.

PROJECT IV: PRESSURE-SENSING MAP KINASE CASCADES IN YEAST AND MAMMALS. Two NSCORT research projects have been initiated: (a) molecular analyses of pressure-sensing proteins in yeast, and (b) pressure-sensing MAP kinase pathways in osteoblasts. The yeast *Saccharomyces cerevisiae* responds to changes in external osmolarity by activating or deactivating one of several pressure sensors in its plasma membrane. To determine how a pressure sensor works, we have chosen the Sho1 sensor for structure-function analysis. The goal is to determine which portions of this membrane protein are necessary and sufficient to sense pressure. Three models for pressure sensing have been proposed. A sensor could sense pressure of extracellular materials on the plasma membrane, stretching of the plasma membrane lipid bilayer, or stretching of the cytoskeleton. There is very little of Sho1 exposed on the outside of the plasma membrane, thus ruling out the former. We have begun making deletions in different portions of Sho1 - transmembrane or cytoplasmic domains - to determine which regions are required for osmosensing. These mutant Sho1 will be tested for function using a yeast strain lacking the SSK2/SSK22 genes that cannot receive a signal from Sln1 and are therefore dependent upon Sho1 for growth in high osmolarity medium. **Pressure-Sensing MAP Kinase Pathways in Osteoblasts:** The function of bone-forming osteoblast cells is enhanced by compression on bone. The mechanism by which compression forces activate osteoblast function is currently unknown, but is likely to involve cytosolic signaling pathways that mediate compression-induced changes in bone cell gene expression. One of the most common mechanisms for mediating extracellular signal-induced gene expression is a MAP kinase pathway, of which there are several known in mammals (e.g., ERK, JNK, p38(HOG)). To investigate this possibility, we have begun an inventory of the MAP kinase pathways present in osteoblasts. More studies are planned to look at the amount of different MAP kinases present at the different stages of primary osteoblast culture development *in vitro*. Following the inventory of MAP kinases, we will then analyze the effect of compression - using a Vitrodyne apparatus - on the 3-D osteoblast cultures, measuring changes in MAP kinase phosphorylation using specific antibodies and changes in stage-specific mRNAs using Northern blots or quantitative RT-PCR.

PROJECT V: LAMINAR FLUID FLOW EFFECTS ON MAMMALIAN CELL PROTEIN SYNTHESIS AND SECRETION. Contrary to the previous belief that a microgravity environment would not affect living organisms at the cellular level, recent findings from the STS-8, SL-1, SL-3, and D-1 missions demonstrate that many cell functions may be altered during space flight. These include increases in growth, changes in size, altered DNA transfer during conjugation, aging modifications, and changes in development and cell differentiation. Specific effects on mammalian cells include a 40-50% increase in kidney cell attachment to microcarriers and a five-fold increase in interferon production by *in vitro* lymphocytes, while lymphocyte mitogenic responses are reduced by more than 90%. Even though prolactin secretion remains unchanged, growth hormone release from pituitary cells is decreased. Mechanisms for these changes are not understood. A consistent problem with much of this work has been the lack of control of many physical and chemical variables and possible nonuniformities in field variables (particularly concentrations). Because of the ability to provide a uniform controlled environment, a stirred-tank bioreactor designed for culture of cells on microcarriers would be ideal for these experiments. However, high agitation rates create hydrodynamic shear and turbulent collisions that result in cell damage and limit the use of fragile cells. Studies on confluent cells in laminar flow chambers have shown that low level fluid shear stress can also alter cell morphology and metabolism, including several-fold stimulation of cell secretion rates. Microgravity experiments need to consider the effects of the hydrodynamic environment within the culture system. We have chosen as a model cell type the human aortic vascular smooth muscle cell (HASMC). Recent modeling studies have shown that HASMC normally experience shear stresses in the range of 1-10 dyne/cm² due to transmural wall flux. Space flight alters pressure distributions within the vasculature, and therefore alters pressure driven transmural wall flux of fluid. If there are significant effects of fluid stress on SMC metabolism and gene regulation, these may be crucial in modulating vascular remodeling during long-term space flight. **Initial Results:** Our first studies were to determine the effects of shear stress on nitric oxide (NO) production by cultured human aortic smooth muscle cells exposed to

increasing levels of shear stress using parallel plate flow chambers. Our findings suggest that human aortic smooth muscle cells express a constitutive neuronal nitric oxide synthase isoform, the enzymatic activity of which is strongly modulated by flow-induced shear stress. This work has recently been accepted for publication in *Circulation Research*. To determine whether shear stress regulates gene expression in vascular smooth muscle cells, we investigated the effect of flow on the expression of the human thrombin receptor (HTR) and tissue plasminogen activator (tPA). Our data indicate that shear stress is capable of regulating gene expression in HASMC and that the HTR promoter contains a shear stress sensitive element. This work has recently been submitted for publication.

PROJECT VI: REGULATION OF G1 CYCLINS AND THEIR CYCLIN-DEPENDENT KINASES DURING T CELL ACTIVATION IN HYPOGRAVITY CULTURE. Studies of lymphocyte (T cell) activation during space flight and in simulated microgravity (clinorotation) show a dramatic reduction of DNA synthesis in response to mitogenic lectins. While some progress has been made in identifying T-cell inhibition in microgravity, a mechanistic understanding of the inhibition is still lacking. In hypogravity inhibition of lymphocytes, the interaction of IL-2 with its receptor, does take place. However, the cells do not exit G1 and fail to initiate DNA synthesis. This finding suggests that hypogravity may alter the regulation of the G1/S checkpoint. Progress to date: The originally stated objectives were based upon observations using the bead-anti-CD3 and PMA/I activation systems. This system usually resulted in entry to the cell cycle (G0/G1 transition) and subsequent arrest at G1/S. We have observed a variability of the G1/S block using these systems that appears to be dependent upon concentrations utilized for the activators. This variability is apparently due to activator concentrations resulting in overstimulation and cell death (with the PMA/I) or induction of anergy (with the bead-anti-CD3). This was a significant problem when basing S phase progression on tritiated thymidine utilization. This assay will look identical for an S phase block or the death of the cells prior to S phase. Therefore, at this time there is some question whether the arrest at G1/S is valid or is an artifact of cell death or anergy. We will continue our efforts to define the experimental system and to remove any influence of potential artifacts.

PROJECT VII: MECHANICAL LOAD EFFECT ON BONE FORMATION. The research plan includes creating a versatile three-dimensional (3-D) polymer/cell construct to model bone behavior under specific mechanical environments. The specific aims are: 1) To create 3-D cultures of primary rat osteoblasts attached on biodegradable polymer scaffolds and to form bone tissue *in vitro*. We will explore the effects of seeding density and foam morphology on osteoblast proliferation, function, and matrix synthesis. We will use the knowledge from these studies to develop culturing protocols for creating our 3-D *in vitro* model with controlled cellularity and bone density; and 2) To investigate with the *in vitro* model the effect of static and cyclical compressive strain exerted on the 3-D polymer/osteoblast cultures for: a) alkaline phosphatase activity, collagen production and gene expression for osteocalcin, osteonectin, and osteopontin, all of which are extracellular matrix components and contribute to mineralization, b) secretion of cytokines and growth factors which play important roles in the bone remodeling process, and c) mineralization and bone formation to understand their adaptation to load conditions. Accomplishments: Bone formation *in vitro* was investigated by culturing stromal osteoblasts in three-dimensional (3-D), biodegradable poly(DL-lactic-co-glycolic acid) foams. This study suggested the feasibility of creating 3-D cultures of primary rat osteoblasts attached on biodegradable polymer scaffolds and forming bone tissue *in vitro*. This study also showed that cell seeding needs to be further investigated for creating cell cultures with controlled cellularity and bone density. We have begun investigations to study cell seeding into porous polymer scaffolds in order to create a 3-D *in vitro* model that better mimics the *in vivo* load conditions. We have developed a new scaffold design and polymer processing method in an attempt to improve cell seeding. To apply strain to the cells within the scaffolds, a new apparatus was designed and built which allows us to treat the scaffolds continuously over a long time period and still support the seeded cells inside the scaffold. This apparatus allows us to control the mechanical load on 3-D polymer/cell constructs including the frequency of the stimulating strain, strain range within a cycle, ratio of strain change, and maximum force applied to the construct. We will use this apparatus with the 3-D cell cultures to study mechanical load effects on osteoblast gene expression and bone formation.

PROJECT VIII: MECHANICAL LOADING, GROWTH FACTOR RELEASE, AND REGULATION OF SKELETAL MUSCLE MASS: A POTENTIAL SITE FOR THE APPLICATION OF MICROGRAVITY-INDUCED MUSCLE ATROPHY COUNTERMEASURES. Understanding microgravity-induced musculoskeletal adaptation, specifically microgravity-induced skeletal muscle atrophy (MISMA), is of critical importance to the future of manned space flight. MISMA, with the consequent loss in muscle mass strength and endurance, is one of the most serious, and potentially dangerous, problems faced by crew members during and after extended space flight. However, the mechanism(s) responsible for the initiation of MISMA are unclear. We suggest that mechanically-induced, wound-mediated release of FGF acts as a transduction mechanism for translating mechanical load into a muscle growth response and that disruption of this mechanism during space flight plays a significant role in the initiation of microgravity-induced muscle atrophy. During the past 12 months, we have completed the preliminary studies outlined in our original proposal utilizing our newly acquired Flexcell Strain Unit (FSU). This work appeared as a research article in the *FASEB Journal* and is the first study to demonstrate a direct, proportional correlation between the amount of mechanical load applied, the degree of sarcoplasmic wounding inflicted on, and the quantity of fibroblast growth factor (FGF) released by differentiated human myotube cultures. In addition, this study demonstrated that the growth response induced by mechanical loading was abolished when the action of FGF, released as a consequence of mechanical loaded-induced sarcolemma wounding, was blocked using a specific, site-directed FGF neutralizing antibody. As such, this work directly demonstrates that sarcolemma wound-induced FGF release is a central signaling mechanism involved in mechanical load-induced skeletal muscle growth. We have recently acquired a second FSU culture platform which will be used for the study of unloading on human skeletal muscle cells.

PROJECT IX: MICROGRAVITY EFFECTS ON LYMPHOCYTE ADHESION AND MOTILITY. This project will focus on the effects of microgravity environments on cells of the immune system. Activation of T lymphocytes requires a specific sequence of cell signaling and intracellular events that are triggered through interactions with monocytes or other antigen-presenting cells (APC). Since migration of lymphocytes and their adherence to other cells are two of the most significant properties modulating these interactions, our research will concentrate on elucidating the effect of microgravity environments on the pathways of lymphocyte adhesion and motility. Measurements of lymphocyte aggregation rates and migration speeds will be performed using two sensitive assays based on video microscopy and digital image processing. Results: During the past six months, we completed the development of a model that describes the kinetics of homotypic cellular aggregation. Such a model is necessary for correctly interpreting the results from lymphocyte aggregation experiments. We have developed a kinetic model and applied it to the analysis of experimental aggregation data. Model predictions agree well with data from homotypic lymphocyte aggregation experiments using Jurkat cells activated by 33B6, an antibody to the b1 integrin. This comparison allowed us to quantify the dependence of aggregation rates on (a) the motility of cells and cell aggregates, (b) the frequency of cell-cell collision, and (c) a measure of the strength of inter-cellular bonds. Thus, this model provides a potentially useful tool for identifying the important physiological parameters involved in aggregation and for correctly interpreting experimental data obtained from visual assays of homotypic cellular aggregation. Plans for the Coming Year: We plan to extend our studies on lymphocyte aggregation and motility to cells that have been cultured in a simulated microgravity environment. These studies will address the following questions: Does exposure to simulated microgravity affect the receptor expression and the binding of monoclonal antibodies to corresponding epitopes of the b1 integrin? Does simulated microgravity affect the aggregation kinetics and do we observe any dosage-dependence effects that can be used to control this adhesive function? Is cell avidity altered only due to conformational changes of the b1 integrin or does ligand-binding induce intracellular activation signals that modulate lymphocyte adhesion function? Using a second assay we developed, we will also evaluate the motility of lymphocytes exposed to simulated microgravity by measuring their speed of locomotion, persistence of movement, and turn angle distribution.

This research will yield a new understanding of basic biological processes. Several of the projects are centered on understanding the molecular basis of the response of various cell types to mechanical stimuli. These projects examine basic cellular mechanisms involved in sensing the mechanical force environment, in transduction of these signals, and in gene regulation. The data produced will have wide application in many areas where mechanical forces are intimately coupled with gene regulation and cell metabolism.

FY97 Publications, Presentations, and Other Accomplishments:

Bamman, M.M., Clarke, M.S.F., Feedback, D.L., Powers, S.K., and Stevens, B.R. "Myosin heavy chain distribution following bed rest with and without resistance exercise." (Abstract) The American College of Sports Medicine, Denver, CO (1996).

Braam, J. "Regulation of expression and functions of XET and calmodulin-related TCH genes of *Arabidopsis*." Symposium on Calcium and Gravitational Biology, The North Carolina Biotechnology Center, Research Triangle Park, NC (November 2, 1996).

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Ishaug, S.L., Crane, G.M., Miller, M.J., Gurlek, A., Yasko, A.W., Yaszemski, M.J., and Mikos, A.G. "Ectopic bone formation by marrow stromal osteoblast transplantation using poly(DL-lactic-co-glycolic acid) foams implanted into the rat mesentery." Student Research Award in the Doctoral Degree Candidate Category, Society for Biomaterials 23rd Annual Meeting, New Orleans, LA (April 30 - May 4, 1997). *J. Biomed. Mater. Res.*, 1-8 (1997).

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Jen, A.C., Almaguer, J.L., Cho, S.D., Widmer, M.S., Mikos, A.G., Akanbi, K., and Farach-Carson, M.C. "Three-dimensional polymer/cell mechanical model for bone formation." Post-ASME Workshop on Cell Biomechanics, Georgia Institute of Technology, Atlanta, GA (November 23, 1996).

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McIntire, L.V. "Differential gene regulation induced by flow in vascular cells: Implications for gene therapy." [Invited Lecture] International Congress on Frontiers in Biomedical Engineering, Tokyo, Japan (July, 1997).

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NSCORT: BIOREGENERATIVE LIFE SUPPORT - Biomass Productivity and Sustainability of Bioregenerative Life-Support Systems

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NOTE: This NSCORT was under no-cost extension during FY 1997.

Funding:

UPN/Project Identification: 199-93-17-03

Solicitation: NRA, 1990, NSCORT Program

Initial Funding Date: 1991

Expiration: 1997

Students Funded Under Research: 1

Post-Doctoral Associates: 3

FY 1997 Funding: \$0

NOTE: An FY 1997 Funding amount of \$0 does not necessarily represent zero money spent on the task for FY97. FY 1997 Funding amounts represent task dollars appropriated from the FY97 Life Sciences budget.

Task Description:

The NASA Specialized Center of Research and Training (NSCORT) in Bioregenerative Life Support at Purdue University from late 1990 through 1995, with a no-cost extension to the end of 1997, provided a center of excellence for training and research related to bioregenerative life-support systems and the construction of a Controlled Ecological Life Support System (CELSS). The participating faculty are experts in technical areas crucial to the development of a CELSS, and all have a distinguished record training graduate students and postdoctoral research associates. Several participants also had previous experience working with NASA in general and with the CELSS program in particular prior to the NSCORT. All participants are comfortable with interdisciplinary collaboration, and all have now worked together.

The major focus of the Purdue NSCORT was the interactive development of crop production, food processing, waste management, and systems integration for a space-deployed CELSS. This was accomplished by an interdisciplinary group with expertise ranging from systems engineering to biotechnology. Recombinant DNA techniques were used to appropriately modify photosynthetic microorganisms and crop plants for the food, atmospheric, and energy requirements of a CELSS. This information and the resulting biomass were utilized to determine appropriate diets for astronauts. Overriding this research was an engineering analysis to optimize the components of CELSS and to ensure that wastes are processed efficiently in future closed life-support systems.

Research in the Purdue NSCORT covered the major elements required for a functioning CELSS. A major and diverse effort focused on biomass production. The goals in this area were focussed on the efficient production of edible biomass, determining the impact of environmental conditions on the quality and quantity of biomass production, providing high quality edible biomass products for food processing, and minimizing waste production. Concurrently, projects determined optimal environmental conditions for biomass production, and how to genetically engineer crops such as rice, brassica, and cowpea for optimal growth and nutritional value. Another project utilized cyanobacteria for production of O₂, for N₂ fixation, for CO₂ assimilation, and for algal

biomass production. In general, our studies of biomass production included an appropriate mixture of basic and applied research with CELSS applications as well as spinoff Earth benefits in mind.

Another major component of the project included research in nutrition and food processing. A primary objective was to convert hydroponically grown, productive plants into acceptable, safe food products. A second objective was to anticipate and provide for human nutritional requirements during long-duration missions or colonization in a hypogravity environment. Another major research area involved renewable resource management and systems engineering. The objective of these projects was to integrate all important subsystems of a functioning CELSS, utilizing three levels of investigation. This research modeled the overall life-support system, identified all essential material items, quantitated these materials and their corresponding flows, and optimized the overall system. Specific areas included engineering for waste processing and process engineering in food production. Objectives of these projects included conversion of waste biomass residue, monitoring of air and water quality, purification of waste water, air-quality improvement, monitoring of biological contamination, bioreactor development, and separation research.

The project contributed new knowledge applicable to an operational CELSS including: more optimal growth conditions for crops and photosynthetic microorganisms; the development of balanced vegetarian diets for CELSS inhabitants; proof of the nutritional benefits of that diet; use of cyanobacteria as a component of CELSS to help stabilize O₂ and CO₂ levels, as well as to provide combined nitrogen; and appropriate ways to stabilize the CELSS environment and processing wastes.

Contributions equal to the research conducted by the Purdue NSCORT included an extensive training program for postdoctoral researchers, graduate students, and undergraduates, as well as a space education outreach program for grades K-12, for civic and educational organizations, and for the general public.

During the no-cost extension period of the NSCORT closeout in 1997, the biomass production laboratory of Dr. Mitchell, the Foods & Nutrition laboratory of Dr. Belury, and the Food Science laboratory of Dr. Floros hosted several trainees finishing up their NASA-sponsored Life Sciences research.

The traditional approach to enhancing photosynthetic productivity and yield of crops grown in controlled environments has been to enhance ambient CO₂ and elevate photosynthetic photon flux (PPF) to saturating levels while keeping other environmental factors non-limiting. Postdoctoral Research Associate Dr. Changhoo Chun compared this "static" control approach of constant high PPF and CO₂ with the "dynamic" control approach of using photosynthetic rate (Pn) as a rapid feedback response to variable increments of PPF and CO₂ over time. Closed canopies of leaf lettuce (*Lactuca sativa* L.) were used as a rapidly growing crop test system with excellent responsiveness to these variables. The dynamic control approach not only varies inputs (i.e., CO₂, PPF) according to preference and tolerance of the crop at that particular time in its development, but also compares increments of output (i.e., ΔPn) and input (i.e., $\Delta kW \cdot h$) and seeks combinations of variables that optimize output/input increment ratios. Dr. Chun modified process-control software used in connection with a computerized photosynthetic measurement system for small canopies. He found that simultaneous dynamic control of CO₂ and PPF gave yield rates of lettuce equivalent to those from static, saturated inputs during the exponential phase of crop production while using only 73% as much electrical energy to power plant-growth lamps. Daily energy-conversion efficiency (g edible biomass / kW-h) for the dynamic control strategy was 132% more than static controls. As they are further optimized, dynamic control strategies hold great promise for even larger savings of crop production input variables.

Another important issue identified by the Purdue Crop Production Group that limits yield efficiency is geometry of plant-growth lighting within closed canopies, especially of planophile (horizontal-leafed) crops lighted from overhead, which includes most dicotyledonous CELSS candidate crop species. Using cowpea (*Vigna unguiculata* Walp.) as a model planophile species, graduate student Jonathan Frantz distributed low-power fluorescent lamps sleeved with transparent mylar heat shields in 3-dimensional space within the headspace of separate growth compartments above a hydroponic growth system. Cowpea canopies were allowed to grow up and around lamps, thereby providing irradiation from many directions within the canopy. Frantz found the

light-scattering properties of white polyethylene film to be superior to the reflective properties of mirrored mylar film as a compartment liner to prevent ambient light from escaping photosynthetic surfaces. Frantz also found horizontal tiers of lamps within compartments superior to vertical columns in terms of resulting crop productivity. Interior leaves reoriented to present their adaxial surface toward the nearest lamp in an array, and flower bud abortion and premature leaf senescence of intracanopy-lighted plants did not occur, unlike with overhead-lighted controls. Frantz found that increasing plant density within a stand between 24 and 97 plants/m² enhanced absolute yield as well as yield rate and improved production efficiency with respect to non-edible biomass penalty. Total edible yield efficiency with respect to temporal, spatial, energetic, and non-edible biomass penalties were not different between 48 and 97 plants/m². He used computer-generated "light maps" (based upon 3-dimensional grids of measured PPF) to predict orientation and distribution of lamp arrays within each compartment. Thus far, Frantz has improved the efficiency of cowpea canopy production rates 130-fold by using low-intensity/low-power intracanopy lighting instead of the usual high-intensity/high-power lighting above closed canopies. Thus far in his study, 45% of the yield obtained from overhead lighting has been achieved with only 10% of the energy using intracanopy lighting as the sole irradiance source. Present efforts include adding lamps to intracanopy arrays in effort to match or surpass yield rates of overhead-lighted canopies at only a fraction of the electrical energy cost.

In Dr. Belury's Foods and Nutrition research laboratory, Postdoctoral Research Associate Kwangok Nickel continued to investigate the nutritional adequacy of CELSS vegetarian diets. In particular she determined the association of nitrogen balance due to dietary protein intake to mineral bioavailability using a rat model. She found that bone health (i.e., strength, flexibility, and density) of animals fed unsupplemented vegan diets was equivalent to that of animals fed control diets. However, overall size of the bones was lower in animals fed vegan diets. Future work in this area should focus on determining how a vegan diet supports protein and mineral status in humans in a microgravity environment

Following is a summary of accomplishments from Dr. Floros' Food Science laboratory, which were part of the studies of Postdoctoral Research Associate Linus Fonkwe: (1) Active packaging was used to extend the shelf life of foods by retarding undesirable chemical reactions. In particular, oxygen absorbers stopped oxidative deterioration by reacting with and removing oxygen from the headspace of packages. The type of oxygen absorber was powdered iron, which oxidizes under certain conditions to form iron oxide. (2) To extend shelf life and retain the quality of fresh fruits and vegetables, antagonistic bacteria and modified atmospheres were used during storage to control fungi and yeast as an alternative to fungicides. Overall, the results showed that modified atmospheres can reduce the growth of *B. cinerea*. Biological control agents such as *Erwinia sp.* were effective antagonists against *B. cinerea* growth on apples, particularly under ambient conditions. However, biological control agents in combination with modified atmospheres were not very effective.

Work conducted by the Purdue NSCORT in Bioregenerative Life Support from 1990 through 1995, and during the 1996-1997 closeout period, primarily addressed the issue of "sustainability" of a bioregenerative life-support systems. The general approach was to identify processes and protocols that would permit development of a recycling life-support system that could operate stably within reasonable constraints of power, labor, mass, volume, and leak rate for long periods of time. Efficiency of candidate processes was an emphasis of research in the Purdue NSCORT.

There are many potential spinoffs from each of the major research projects of the Purdue NSCORT, but the relatively brief window of funding has precluded development to a useful end point. The nature of the potential spinoffs is detailed in a publication co-authored by Purdue NSCORT faculty and their UC Berkeley collaborator (Mitchell et al. 1996. Earth benefits of interdisciplinary CELSS-related research by the NSCORT in bioregenerative life support. *Adv. Space Res.* 18(4/5):23-31). Briefly, Earth benefits from continuation of this NSCORT would have included the following: development of dynamic optimization systems for the energy-efficient control of light, CO₂, and temperature in the hydroponic, controlled environment production of CELSS candidate crop species; guidelines for using cyanobacteria to maintain or readjust O₂, CO₂, and N₂ balance within a complete CELSS system; development of transgenic cereal and legume crops no longer deficient in essential amino acids and needed for a protein-balanced vegetarian diet; use of anti-sense RNA

technologies to create low non-edible crop residues during crop production; learning how to control nutrient/anti-nutrient/toxin contents of edible crop biomass in a useful way by manipulating crop production environments and mineral nutrition; development of food-process procedures to remove/destroy anti-nutrients from crop biomass; development of food-process technologies and equipment for use in non-extensive closed systems; development of rapid microorganism-based technologies for bioconversion of organic wastes to renewable resources or intermediate, novel food products; and development of control system strategies for complex systems that otherwise have potential for chaotic behavior of subsystem components. Although publications still coming out from Purdue NSCORT work in each of these potentially valuable spinoff areas are benchmarking progress made toward realizing these goals, the loss of interdisciplinary synergism will greatly increase the timeline and expense required for their eventual realization.

FY97 Publications, Presentations, and Other Accomplishments:

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Mitchell, C., Chun, C., Brandt, W., and Nielsen, S. "Environmental modification of yield and nutrient composition of 'Waldmann's Green' leaf lettuce." *J. Food Quality*, 20, 73-80 (1997).

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Stephens, S., Watkins, B., and Nielsen, S. "Storage stability of screwpress-extracted oils and residual meals from CELSS candidate oilseed crops." *Adv. Space Res.* (In Press).

NSCORT: Vestibular Research/(NIH)

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NOTE: This NSCORT represents five individual tasks with five principal investigators.

Funding:

UPN/Project Identification: 199-93-17-09
 Initial Funding Date: 1993
 Students Funded Under Research: 4
 FY 1997 Funding: \$500,000
 Joint Agency Participation: NIH

Solicitation: not available
 Expiration: 1998
 Post-Doctoral Associates: 1

Task Description:

This Center is designed to define the contributions of the vestibular system to the control of balance, posture, and locomotion through an integrated series of ground-based studies, three examining the vestibular-neck (vestibulo-colic) reflex and two the vestibulo-spinal control of standing posture. One theme of the Center is to exploit the synergy between these two sets of studies to produce the first complete whole body model of posture. Any model that is to lead to an adequate understanding of the postural system must incorporate and interrelate mechanisms that stabilize the head in space, the trunk with respect to the head, and body center of mass with respect to gravity. Heretofore, no investigator or group has had the broad array of skills and insights to attempt such a model or to undertake the interactive experiments needed to obtain the data upon which it must be based. This Center will provide the skills and resources to accomplish this important task, which the field has been awaiting for a long time.

The second theme of the Center is to focus upon the vestibular otolith organs and the sensorimotor responses that occur when they are stimulated by gravitational forces or linear motions. Projects One and Two bring on line new devices designed specifically to study otolith systems. Modelers involved in Projects One, Two, Four, and Five simulate and model for the first time the role of neural pathways originating in otolith organs in stabilization of the head and body and in locomotion. Collectively, these activities will greatly increase our knowledge of otolith systems, which are of special importance for understanding how the neurovestibular system senses and adapts to the alteration in gravity that occurs when a spacecraft enters orbit and returns to Earth with attendant problems of disorientation and dysequilibrium.

A third theme of the Center is its extensive use of computational modeling. Projects One, Four, and Five share the use of an elegant new biomechanical model that allows one to construct accurate models of musculoskeletal systems whose kinetic properties can then be simulated under a wide variety of conditions. Projects One and

Five employ non-linear systems models to simulate how central nervous system control of head or body position interacts with body biomechanics. Our goal is for these modeling efforts to coalesce into a multi-level model that both simulates postural stabilizing responses observed by us and others and suggests further experiments that will more effectively illuminate the functions of the vestibulo-spinal system.

The Center also has a training component designed to give pre- and postdoctoral trainees unique opportunities to work with outstanding vestibular physiologists and modelers and to participate in work on several Center projects, thus contributing to the cross fertilization taking place within the Center.

Considerable progress has been made by each of the five projects supported by this center.

PROJECT 1: ANGULAR AND LINEAR VESTIBULOCOLLIC REFLEXES IN HUMANS. We have now collected closed loop VCR and CCR in 10 subjects with complete bilateral labyrinthine loss. The data show that there is a great deal of diversity of responses of these patients, presumably reflecting different degrees of adaptive recovery and different recovery strategies employed by the patients. We have also continued studies examining the dynamic properties and behavioral modulation of the linear VCR using our linear sled employing a sliding frequency, anterior-posterior linear velocity "Chirp" stimulus. The expected resonance was not found indicating that the system has behavioral elements that remain to be characterized.

A series of measurements of head forces and related neck muscle EMG activity have been made in 10 normal subjects as a step in validating our biomechanically accurate model of the human head-neck system. Data support our conclusion that the classically defined set of neck muscles is inadequate to account for observed maximal flexion torques that humans can produce. Our EMG recordings show that both infra and supra-hyoid muscles are strongly activated when such torques are generated and the model indicates that due to their large moment arms, these muscles can account for the "missing" torque.

PROJECT 2: LINEAR VESTIBULOCOLLIC REFLEX IN PRIMATES. We are progressing in our studies of electromyographic responses during reflex excitation of neck muscles during externally applied linear and rotational motions. The focus continues to be upon the spatial reorganization of squirrel monkey vestibulocollic reflexes when the body is re-oriented with respect to gravity. The salient finding is that the direction of neck muscle excitation reverses when the head is inverted with respect to gravity. This means that the direction of the vestibular neck reflexes is reversed so that they become anti-compensatory in the inverted head posture. Two possible, related explanations of this result are being explored. First, it could be that the compensatory vestibulo-collic reflex which operates in the normal upright posture competes with a positive-feedback anti-compensatory righting reflex when the posture is far from the normal position. Second, it could be that the stability of the head provided by gravity when the head is inverted is accompanied by shut-down of the then unnecessary stabilizing reflex. Instead, when the head is inverted it may be too stable (too hard to move into a different posture), and a reversed reflex is needed to set head stability at a level similar to that in the upright posture. Our data now point in the direction of a otolith driven righting reflex which dominates head movement behavior under the conditions of these experiments.

PROJECT 3: HEAD STABILIZATION IN THE MONKEY. Significant progress has been made during the last year. We now have over a hundred neurons of different types that have been observed during active and passive head movements. Over 80% of these show a suppression of their vestibular responses when an active, voluntary movement is made. Two full-length papers are now being readied for publication which will make the following points: 1) the gain changes in the vestibulo-collic reflex are linked to active gaze pursuit, and 2) these are the result of disparate signals on vestibular neurons, including identified vestibulospinal neurons, during externally applied or passive and self-generated or active head movements. In particular neck afferent inputs can account for all or part of the suppression in about a third of the neurons while suppression of vestibular responses in the others must be due to centrally generated cancellation signals.

PROJECT 4: ORGANIZATION OF POSTURAL CONTROL: VESTIBULAR PROCESSING. This project aims to identify the contribution of otolith and canal signals as well as biomechanical constraints to postural

control strategies. This year, we have applied our understanding of postural coordination in healthy subjects to patients with severe, bilateral vestibular loss in order to relate otolith and canal function to specific aspects of postural coordination. In collaboration with Dr. Peterka, we found that patients with severe, bilateral loss of horizontal canal vestibular function could show relative preservation of otolith and vertical canal function as tested by measurement of vestibuloocular reflexes with off-vertical axis and pitch plane rotations. Postural coordination was shown to be abnormal in these patients with loss of vestibular function in two tasks: 1) selection of a hip strategy in response to large, fast surface translations when vestibular loss occurs early, but not late, in life; and 2) control of the trunk orientation and stability during sinusoidal oscillations of the support surface with eyes closed.

Contrary to our earlier hypotheses, we found that vestibular function is not necessary to trigger a hip strategy but it may be critical during infancy to establish normal hip patterns. We also report similarities and differences in biomechanical and neural constraints on postural control in the sagittal and frontal directions. Sagittal and frontal postural responses showed similar kinematic and surface force strategies but different muscle synergy patterns with early trunk control for regulating lateral posture and early ankle control for regulating sagittal posture. The critical role of somatosensory information in triggering postural responses was revealed in a very rare patient with total body somatosensory loss since automatic responses both to external and to self-generated perturbations were absent. Nevertheless, this patient was able to stand independently and resist perturbations by longer-latency substitution of auditory and visual cues when perturbation direction was predictable.

We are also expanding our model of postural control to include a process for adaptation of postural control for altered sensory environments. The model suggests that use of an internal, neural model of expected sensory information, which may relate to cerebellar function, can be used to predict postural behavior under altered sensory conditions. Multivariate descriptors of human postural sway are useful to fully characterize postural stability under altered sensory conditions and are being tested in patients with either vestibular loss or somatosensory loss from diabetic peripheral neuropathy.

PROJECT 5: VESTIBULOSPINAL CONTROL OF POSTURE AND LOCOMOTION. The aim of this project is to investigate the role of the vestibular system in maintaining balance during stance in the cat. The focus is on stabilization of the head and trunk during quiet stance and during responses for postural equilibrium. The EMG and modeling studies of the vertebral column were revised and 2 papers are now in press. Based on these results, we have now begun to examine the putative antigravity muscles of the scapula and ventral abdomen. These muscles all show the tonic activity expected of antigravity muscles. The abdominals are particularly interesting because they behave dynamically as flexors even though they have antigravity function. A second study was completed this year concerning the effect of static head position on postural orientation and the response to stance perturbations. This study addressed the issue of sensorimotor transformations in terms of the mapping between vestibular and neck afferent inputs and the rapid motor response to maintain postural equilibrium. Large shifts in head position involve not only the head/neck system but also the anterior trunk and scapulae, particularly in the yaw axis. Head position appears to have little or no affect on dynamic equilibrium, suggesting that different combinations of vestibular and neck afferent inputs characterizing the various head positions always mapped onto the same motor output space. This study is an important prelude to examining the effect of dynamic head motion on posture and balance and has important implications for spatial orientation.

Projects One and Four, which examine vestibular reflexes in humans, are yielding information that will help us understand and treat disorders of balance and posture. Proper control of the head-neck motor system during rotations and translations of the body is essential for controlling the orientation of the head's special sensory receptors in space and regulating the attitude of the head on the trunk as part of overall postural control. Such control can be seriously degraded in patients with vestibular or neurological abnormalities. Work related to Project One is helping us to understand the problems experienced by such patients and is suggesting ways in which they could be ameliorated. This project is providing the first information available on otolith-driven vestibular-neck reflexes.

It is not yet possible for clinicians to accurately diagnose disorders of the vestibular otoliths in humans since their role in vestibulo-spinal behavior is unclear. Studies carried out under Project Four suggest that vestibular

information, particularly otolith information, may be critical for control of specific types of postural tasks under specific sensory and biomechanical constraints on balance. Tasks which require dynamic head and trunk orientation, however, may require accurate vestibular input. One goal is to be able to predict the postural tasks likely to be difficult or impossible for patients with loss of vestibular otolith and/or canal function which may also apply to astronauts in space. A better understanding of the role of vestibular information in postural control will lead to improved diagnosis and rehabilitation of balance problems in vestibular patients as well as avoidance of unnecessary problems in astronauts. It now appears that vestibular patients may have selective loss of the canal or otolith components of their vestibular sensory input. Development of our new, sensorimotor computational model of human postural control has the potential to predict the effects of altered sensory and/or biomechanical conditions (either from disease or extraterrestrial conditions) on postural coordination and stability.

In addition, work related to Aim four of Project One is making good progress toward obtaining the first biomechanically accurate model of the human head-neck system. Given the great interest in this system from the standpoint of human factors and whiplash studies, it is surprising that no such model exists in the scientific literature to date. Indeed, it has been found that additional muscles must be added to those traditionally thought to control head movements in order to account for human motor performance. Our model thus has a wide application in a variety of disciplines that heretofore have relied on crude approximations of head-neck biomechanics to understand the dynamic behavior of the human head.

Projects One and Four are also providing information on basic biological processes involved in converting input from receptors of the vestibular labyrinth into motor commands required to maintain postural stability. A wealth of additional basic biological information is being generated by Projects Two, Three, and Five, which examine vestibulo-spinal systems at levels of detail that are not possible in humans. Projects Two and Five are revealing the detailed patterns of reflex muscle activation that underlie postural stabilization. Project Three is revealing exciting new aspects of central neural processing that allows neurons of the vestibulo-spinal system to differentiate between vestibular afferent signals generated by voluntary head movements and passive displacement of the body — an attribute of CNS processing that is likely critical for accurate postural regulation.

With their common emphasis on processing of vestibular afferent signals, especially those arising from gravity-sensitive otolith organs, these five projects also have obvious relevance to space flight. Under microgravity conditions on orbit, the reflex stabilizing systems we are studying must be modified to maintain proper motor control. Such modifications must then be reversed upon return to Earth. Residual adaptive changes likely account for many of the postural problems experienced by astronauts immediately after landing. Our results should help to understand and remediate these problems.

FY97 Publications, Presentations, and Other Accomplishments:

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NSCORT: The Center for Gravitational Studies in Cellular and Developmental Biology

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Task Description:

The proposed focus of this Center is in the area of gravitational biology with the research emphasis in cellular and developmental biology. The research component of the Center has been developed in light of the existing information available on the role of gravity in cellular and developmental biology. It is already clear that reduced gravity has a significant impact on some cell types and cellular activities, and on some developmental systems and processes. However, a comprehensive and focused analysis has not yet been conducted. The individual projects proposed for this Center are diverse in that they include studies on higher plant, protozoan, yeast, insect, avian, and mammalian systems. The particular feature of the research effort that relates to such diversity is our unifying hypothesis that the cellular cytoskeleton and the extracellular matrix (ECM) represent gravity sensitive macromolecular assemblages. When viewed from the standpoint of this hypothesis, the diversity of the research systems being used becomes particularly advantageous as a comprehensive approach to analysis of the impact of gravity on cellular and developmental biology. It is clear that the plasma membrane is central to the regulation of cellular activities. Thus, incoming signals, whether from distant sites (like hormone or growth factor ligands) or from the local environment (like ECM molecules), bind to integral membrane receptors. Signal transduction events routinely involve the cellular cytoskeleton, either directly or indirectly. The cytoskeleton is composed of several dynamic macromolecular assemblages crucial to control of cell division, cell motility, cell shape, and endo- and exocytosis. Thus, the cytoskeleton is crucial to the cellular processing of incoming information, which leads to the generation of a cellular response. When the response involves morphogenesis, differentiation, or cell division, the cytoskeleton is again centrally involved. If secretory activity or exocytosis is part of the response, the cytoskeleton is essential. Therefore, the cytoskeleton also controls outgoing information. Furthermore, secretions into the local environment modify the ECM both by assembly and degradation. In both cases, the altered ECM represents a new set of signals to interact with receptors on the cell surface, producing yet another cellular response. The ECM, therefore, is a dynamic macromolecular assemblage whose state and degree of organization is also crucial to cell motility, cell division, morphogenesis, and differentiation. The plasma membrane, which houses receptor and signal transduction proteins, is the intermediary between the proposed gravity sensitive intracellular cytoskeletal compartment and extracellular ECM compartment. Each of the projects proposed for this Center addresses some aspects of this system and will serve to elucidate our understanding of gravity in cellular and developmental biology. The unique strength of the research component is in the combination of our unifying hypothesis and our selection of faculty

scientists and projects with diversity, breadth, and depth that will ensure a systematic and serious test of that hypothesis.

The research conducted in this Center addresses fundamental questions regarding the role of gravity in cellular and developmental biology. The progress made on specific research projects has substantial potential value to humankind on Earth, as well as to a manned presence in space. While the research is basic in nature, there are enormous potential benefits. Some examples of areas of impact include:

Immune cell biology: These studies are of value in understanding the immune system in normal and compromised situations, as experienced both on Earth and in space, and have potential in understanding of immune cell interactions, cytokine production and regulation, and potential therapies for correction of disease states and altered physiological states.

Plant developmental biology: These studies have potential impact in the general areas of agriculture and food production and quality, and physiological understanding of gene regulation in harsh environments, such as closed systems, non-optimal gas, light, temperature, and gravity situations, as can be found on Earth and during space flight.

Eye development: This research impacts on understanding the structure, function, development, and gene regulation in the vertebrate eye, and has major potential benefit in understanding of, and possible therapies for, various eye diseases, including cataracts, keratoconus, and optic dysfunction.

Embryonic organ development: These studies have potential impact on understanding of abnormal development and birth defects, and specific disease and dysfunctional organ situations, including lung (respiratory distress syndrome), pancreas and salivary glands (digestive enzymes, exocrine function, endocrine function, diabetes), heart formation (congenital defects, myocardial disease, circulation), and skeletal tissue formation (osteoporosis).

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