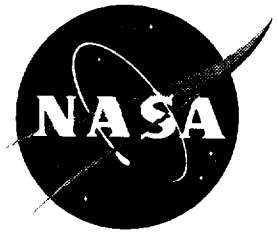
NASA Technical Memorandum 4751



Life Sciences
Program Tasks and Bibliography for FY 1995

Office of Life and Microgravity Sciences and Applications

Life Sciences Division
Code UL

National Aeronautics and Space Administration
Washington, D.C. 20546

June 1996

Euro-Mir

Ground Definition

Life and Microgravity Spacelab (LMS)

NASA-Mir-1B

Neurolab

Spacelab-Mir (SLM-1A)

Space and Life Sciences-2 (SLS-2)

Small Payloads

Ground-based Programs:

Advanced Life Support

Advanced Technology Development

Data Analysis

Environmental Health

Global Monitoring & Disease Prediction

NSCORT

Space Biology

Space Human Factors

Space Physiology & Countermeasures

Space Radiation Health

I.
Introduction

TASKBOOK INTRODUCTION FOR FY1995

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. Flight tasks are currently organized by payload complement or program while ground-based tasks are divided into ten major scientific programs: Advanced Life Support, Advanced Technology Development, Data Analysis, Environmental Health, Global Monitoring and Disease Prediction, Space Biology, Space Human Factors, Space Physiology & Countermeasures, Space Radiation Health, and the NASA Specialized Centers of Research and Training (NSCORTs).

FY 1995 proved to be an important year for the Life Sciences Division. The Division released two NASA Research Announcements (NRAs). Investigators chosen from the latter NRA will fly aboard the United States Space Shuttle and the Russian space station *Mir*, serving as a first stepping stone to planned investigations aboard the future International Space Station. The Space Shuttle made its first historic linkup with the Russian space station in June of this year, ushering in a new era of joint international space exploration and promising future research into the effects of long- term space exposure on terrestrial organisms.

This document, the Life Sciences Program Tasks and Bibliography for Fiscal Year 1995 (October 1994-September 1995), includes all peer reviewed projects funded by the Office of Life and Microgravity Sciences and Applications, Life Sciences Division, during that year. Additionally, this inaugural edition of the Task Book has also included FY 1994 citation information for historical purposes. This document will be published annually and made available to scientists in the space life sciences field both as a hard copy and as an interactive internet web page (https//www.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.

The Life Sciences Division wishes to thank Information Dynamics, Inc., and Universities Space Research Association personnel at NASA Headquarters and in particular recognize Mr. David Reed (task book review process and publication manager) and Mr. Bill Wilcox (data system development) for their lead 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 and Laura Lewis at ARC; Dr. Jerry Homick, Elisa Allen, Sharon Jackson, and Bonnie Meadows at JSC; Bill Knott, Debbie Vordermark, and Ray Wheeler at KSC.

FY1995 PROGRAM RESEARCH TASK SUMMARY: Overview Information and Statistics

Total Number of Principal Investigators: **261**

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Total Number of Tasks: **334**

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Total Number of Bibliographic Listings (FY1995 only): **1319**

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Number of Students Funded: **906**

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Number of States with Funded Research (including District of Columbia): **40**

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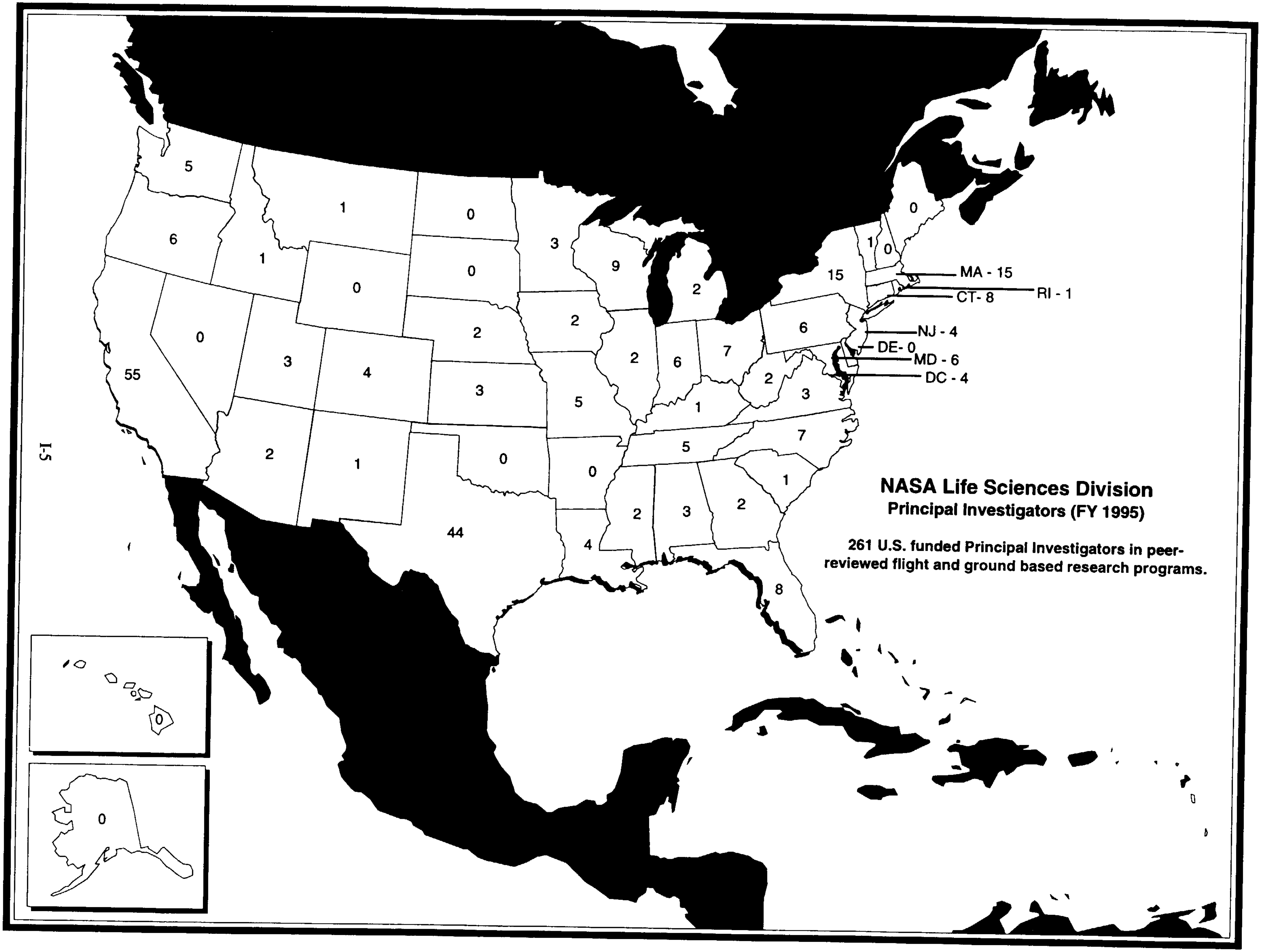
FY 1995 Life Sciences Budget: ..... **$187.3 Million**[1]

Life Sciences Flight Tasks — Listed by Program—		*Life Sciences Ground Tasks — Listed by Program—*	
Program		**Program**	
Bion	9	Advanced Life Support	12
Biorack	9	Advanced Tech. Demo	6
Biospecimen Sharing	7	Data Analysis	9
Cosmos 2229	1	Environmental Health	6
Euro-Mir	12	Global Monitoring and Disease Prediction	1
Ground Definition	3	NSCORT	7
LMS	13	Space Biology	42
Mir-1B	17	Space Human Factors	11
Neurolab	22	Space Physiology & Countermeasures	69
SLM-1A	26	Space Radiation Health	17
SLS-2	2		
Small Payloads	33		
TOTAL	**154**		**180**

[1]This figure includes $46.8 million for space station facilities development.

NASA Life Sciences Division
Principal Investigators (FY 1995)

261 U.S. funded Principal Investigators in peer-reviewed flight and ground based research programs.



II.
Life Sciences
Program Tasks for FY 1995

- **Flight Tasks**

- **Ground Tasks**

Velocity Storage In Space: Adaptation of Optokinetic Nystagmus and After-Nystagmus to Microgravity

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Mingjia Dai, Ph.D. — Mt. Sinai School of Medicine
Sergei Yakushin, Ph.D. — Mt. Sinai School of Medicine

Funding:

Project Identification:

Solicitation:

Initial Funding Date: 1/95

Expiration: 12/95

FY 1995 Funding: $161,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion

Responsible NASA Center: Ames Research Center

Task Description:

In space, the otoliths constantly sense only a fraction of gravitational force and momentarily receive only small amplitude linear accelerations during head translations. Thus, it might be expected that otolith-ocular reflexes that are mediated by the linear vestibulo-ocular reflex (lVOR), such as ocular counter- rolling (OCR) and ocular vergence that orient the eyes to gravity, would be depressed after adaptation to microgravity. In accord with this, the amplitude of two otolith-ocular reflexes, ocular counter-rolling (OCR) and ocular vergence, were reduced for 11 days after the COSMOS 2229 space flight in two flight monkeys. There also tended to be a reversal in the up-down asymmetry of vertical nystagmus, and a shift of the spatial orientation axis of velocity storage, known as the yaw axis eigenvector, toward the body axis. Thus, otolith-ocular reflexes that orient the eyes to gravito-inertial acceleration (GIA) were changed over relatively long periods of time after reentry. Changes in the orientation of velocity storage and in the up-down asymmetry also occurred after space flight, but were of shorter duration, lasting only several days after recovery.

However, steady state horizontal eye velocity, induced by yaw axis off-vertical axis rotation (OVAR), was not different before and after space flight, nor was there a change in the phase of the torsional component of the OVAR response. Vertical and horizontal ocular compensatory responses produced by the angular vestibulo- ocular reflex (aVOR) were also unaffected. The latter indicates that the brain was able to respond to linear acceleration sensed by the otoliths to generate an eye velocity signal from velocity storage correctly after space flight, and that rapid compensatory movements from the aVOR were unaltered. (The Russians have previously shown that the aVOR is altered during active head and

eye movements in space. Presumably, the difference in results represents a difference between the voluntary and passively-induced aVOR.

The purpose of this research is to study how spatial orientation of the linear and angular vestibulo-ocular reflexes (lVOR and aVOR) of monkeys are altered by space flight. We will use eye movements produced by or dependent on the otolith organs and the semicircular canals as the measures of this orientation. We will also investigate effects of gravito-inertial acceleration (GIA) on the angular VOR (aVOR), through a study of velocity storage. Changes in the aVOR during active gaze shifts that involve head and eye movements will be recorded in space by the Russians and compared to the aVOR recorded during passive rotation on earth by US scientists. Finally, we will use binocular three-dimensional recordings in our ground-based recordings to enhance our understanding of how eye movements are affected by changes in the GIA before and after space flight.

We postulate that otolith-induced or dependent eye orienting responses that tend to align the eyes to gravito-inertial acceleration (GIA), will either be reduced after space flight, as for OCR and vergence or as for velocity storage, will be shifted to align with a body axis. Semicircular canal-dependent compensatory responses, on the other hand, will be largely unaffected. Active gaze shifts that involve head movements in space will be altered, but there will be no changes in the passive aVOR recorded on Earth.

For the upcoming BION flight, we will take our laboratory to Moscow in April/May of 1996 and test a control group of 12 rhesus monkeys. They will receive rotation around a vertical axis, rotation around axes tilted from the vertical and optokinetic stimulation with the animals upright and tilted with regard to gravity. During the summer, two of the animals from the group of 12 will fly in the BION space capsule for approximately two weeks. While in space, their bodies will be restrained, but their heads, arms and legs will be free. They will perform behavioral tasks, moving their head and eyes toward lateral visual targets and will press a light with their hands to receive a food or water reward. While in space, the monkeys will be tested for spontaneous nystagmus using electro-oculography (EOG). Their general status will be monitored with down-linked video. At the end of the flight, the space capsule will be parachuted to the earth, and the animals will be recovered by helicopter. They will be kept in their chairs and held in darkness to the extent possible, while they are flown back by jet to Moscow for testing. Postflight testing will extend for 14 days at the Institute of Biomedical Problems. All ground-based testing will be done with binocular three-dimensional eye coil recordings. At the conclusion of the recordings, the equipment will be returned to our laboratory in New York, and the flight data will be processed.

Our hypotheses include the following:
A. Otolith ocular reflexes induced through the lVOR, such as ocular counterrolling (OCR), the horizontal and vertical lVOR and ocular vergence, will be reduced for 5-7 days after space flight.

B. The orientation of the yaw axis eigenvector of velocity storage in the vestibulo-ocular reflex (VOR) will shift from a gravitational to a body yaw axis as a result of adaptation to microgravity. This will be apparent when animals are tested on Recovery Day 0 and will quickly recover.

C. The passive aVOR will be unaffected by space flight, although the active aVOR, manifest during lateral gaze shifts in space, will show changes.

D. Listing's Plane will be unaffected by adaptation to space, but there will be disconjugate vertical and torsional eye movements after space flight.

In the future, there will be intense activity necessary to prepare for the flight. This includes purchase of equipment, programming to accept six channels of eye movement data in our data collection programs, purchase of hardware for the surgical implantation of head mounts and eye coils. Then we will pack the equipment for shipment to Moscow and undertake the actual stages of preflight implantation and

recording. In preparation for this mission, we have been doing baseline data collection and finishing analysis of the COSMOS 2229 flight to the appropriate data base for the BION flight.

We are currently doing baseline data collection in monkeys implanted with eye coils that record eye position in three-dimensions to study changes in eye velocity and in the axis of eye orientation during steps of linear acceleration utilizing binocular recordings of horizontal, vertical and torsional eye position.

We published the following results from the 1992-1993 COSMOS 2229 Mission. Ocular counter-rolling (OCR) was decreased by 70% after space flight in both static and dynamic tests of otolith-ocular reflexes. The reduction in OCR lasted for 11 days, the period of post-flight testing. This is the first time that it has been demonstrated that there is a clear and long lasting effect of space flight on otolith-ocular reflexes. The orientation axis of velocity storage changed from a gravitational reference before flight toward a body reference after flight. This is consistent with our results in the COSMOS 2044 flight (Cohen et al. 1992), and it supports the hypothesis that there is a change in spatial orientation from a gravitational to a body frame of reference in space. In control testing using off-vertical axis rotation, it was shown that there is a modulation in vergence that accompanies the change in gravito-inertial acceleration along the naso-occipital axis. This is the first report of an effect of linear acceleration on ocular vergence during OVAR in the monkey (previous reports were by Tomko and Paige during linear acceleration using a sled). It provides a new, robust and relatively simple technique for testing otolith-ocular function at various levels of gravitational acceleration. Vergence associated with linear acceleration during OVAR was greatly attenuated after the 2229 Mission for a prolonged period after flight (>11 days). This finding has implications for visual function in space, since vergence during forward translational movements would be absent or attenuated in space.

We reviewed our eye coil data to demonstrate that in our laboratory eye coils have been very stable. In 5 monkeys we determined that the horizontal and roll coils had been in place without revision since they were implanted in 1993 and 1994. This was almost two years for three of the animals and one year for the other two animals. Based on this, we conclude that our eye coil technique will be suitable for use in the upcoming BION flight.

We are upgrading the primate axis drive motor on the 3 axis COSMOS rotator to provide smooth controlled acceleration during velocity steps and for doing sinusoidal linear acceleration. New software is being written to collect and analyze 6 channels of eye position data (horizontal, vertical & roll for both eyes). A new eye coil apparatus has been purchased from Neurodata to provide 6 simultaneous eye coil recordings (horizontal, vertical and roll position for each eye). This equipment is now being tested and it will permit analysis of the signals from both eyes during testing.

Equipment status is a critical factor in preparing for the flight. Much of the current equipment is old or not appropriate for use in this flight. The following are the status of the equipment that is being upgraded: Computer equipment from the last flight is obsolete and cannot be used for this mission. The following upgrades will be accomplished: A Pentium PC will be used to run the experiments and take and analyze the data. Data will be stored on Read/Write CD ROMS. Other modifications will be made to upgrade the computers that are used for analysis and provide backup for the equipment that will go to Moscow. Electronic filters, stereotaxic apparatus and surgical equipment will be purchased for use in the Moscow flight.

When our new computer programs are available for taking binocular eye position data in three dimensions, we will study the linear VOR (LVOR) of normal monkeys during OVAR and sinusoidal linear acceleration and determine the characteristics of horizontal eye velocity modulation as a function of head position with regard to gravity and also the relationship of vergence to gravity.

Surgery: Sergei Yakushin, Victor Rodriguez and Bernard Cohen will travel to Moscow in May, 1996, for 14 days to implant head rings and eye coils. They will use a stereotaxic apparatus that will be

purchased for this purpose. Preflight testing and data analysis will be done by a team led by Mingjia Dai. This team includes Leigh McGarvie, Sergei Yakushin and Victor Rodriguez. Bernard Cohen will oversee some of the data taking, and Nicholas Pasquale will come briefly to help Leigh McGarvie set up the laboratory at the IBMP. Postflight testing: Postflight testing and data analysis will be done by the same team as above. Again, Bernard Cohen will oversee some of the data taking, and Nicholas Pasquale will come briefly to help Leigh McGarvie pack the equipment for return to the US.

The proposed research will determine how otolith-ocular reflexes are altered after adaptation to space. In particular, we will show how ocular counter-rolling, ocular vergence and spatial orientation of the angular vestibulo-ocular reflex (aVOR) are altered after adaptation to microgravity. This information, obtained from monkeys whose oculomotor and vestibular systems are similar to those of humans, will be used to understand deficits in gaze and posture that occur when astronauts adapt to microgravity and then readapt to the 1g 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 when long duration space flights are planned to the Moon or to other planets.

Basic information is being developed in this proposal. A major advance will be a three-dimensional model of the vestibulo-ocular reflex (VOR) which will include both angular and linear acceleration inputs, and will account for dynamic changes that alter the orientation of the system vectors to those of gravito-inertial acceleration. In addition, the proposed experiments will provide fundamental understanding of how processing of otolith information and spatial orientation are altered in the absence of gravity.

A basic assumption of the research is that findings obtained during and after space flight can be explained as parameter changes in processes by which the nervous system controls gaze and posture. Therefore, findings from space research can readily be applied to human disorders on Earth. Specifically, we hope to gain understanding of how spatial orientation is disrupted in conditions in which there is postural imbalance or gaze instability. A simple example of the former is postural imbalance of the elderly. We anticipate that information gained from changes after adaptation to prolonged weightlessness will help us understand the imbalance of the elderly.

New technology will be utilized in the three-dimensional analysis of eye movements. This technology will be applied to the recording and analysis of eye movements in three-dimensions by video techniques in humans. It has large potential clinical significance.

A complete model of the VOR would be extremely useful and could be applied to understanding processing in the vestibular system for experiments in animals, and for understanding effects of lesion in the vestibular system and cerebellum in humans.

The neural coding used by the nervous system in establishing spatial orientation of the VOR is not known. The work in this project will provide basic information about how the parameters of the system change with regard to the body when gravitational force is absent. This will help establish how gravity and gravito-inertial acceleration (GIA) are coded in the nervous system and how they are expressed through the VOR. A number of fundamental behavioral and modelling papers have already come from this work. By utilizing data from the BION project, we anticipate that additional insights as to how the GIA is coded will become apparent.

Publications, Presentations, and Other Accomplishments:

Dai, M., Cohen, B., Raphan, T. "Ocular Vergence Induced By Off-Vertical Axis Rotation (OVAR) Before and After Spaceflight." Neurosci. Abstr., 21, 137, 1995.

Dai, M., McGarvie, L., Kozlovskaya, I.B., Raphan, T. and Cohen, B. "Effects of spaceflight on ocular counterrolling and spatial orientation of the vestibular system." Exp. Brain Res., 102, 45-46, 1994.

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Functional Neuromuscular Adaptation to Spaceflight

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Dr. Roland Roy — University of California, Los Angeles
Dr. Richard Grindeland — NASA Ames Research Center
Dr. John Hodgson — University of California, Los Angeles

Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1995 Funding: $299,964

Solicitation: NRA 88-OSSA-8

Expiration: 12/95

Students Funded Under Research: 23

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

Responsible NASA Center: Ames Research Center

Task Description:

There are 2 scientific components of our project with both components using the same technical approaches. Those components are: 1) Adaptive properties within and among motor pools associated with movement of the ankle during well defined motor tasks and 2) Role of muscle activity and muscle force in maintaining normal muscle properties. We have reasonable evidence that some of these fundamental properties of recruitment strategies will be modified by the chronic absence of gravitational forces.

In the next 2 flights, i.e., Bion 11/12, we are in an excellent position to answer rather conclusively and elegantly the degree to which the absence of gravity and /or load bearing of the lower limbs induces changes in recruitment strategies of motor pools during spontaneous, as well as during well defined motor tasks.

Regarding the second science component of our project we also are well prepared. Although it is clear that some muscles atrophy and become weaker as a result of space flight in humans, rats and apparently monkeys, we do not know why. A logical hypothesis is that skeletal muscles must have some "normal" level of activity to maintain normal muscle properties. A second hypothesis is that the force generated by a muscle is directly related to muscle fiber size. A third hypothesis is that hormonal deficiencies, such as growth hormone, contribute to a loss of muscle mass. Probably all three of these factors contribute in a complex interactive way to the atrophy that occurs in space flight, but no one

has quantified any of these potential stimuli in space flight in a way that has been useful in defining their role in maintaining muscle mass. The responses of a series of muscles will be extensively quantified using a variety of assessment tools by an international team of scientists. Muscles of the leg, arm and neck will be biopsied. EMG data will be obtained from the soleus, medial gastrocnemius and vestus literalis, all of which will be biopsied by our colleague Dr. Sue Bodine-Fowler. Muscle force will be obtained from the medial gastrocnemius tendon. Plasma growth hormone will be analyzed by Drs. Vailas and Grindeland with whom we have maintained a close collaboration in several ground based and several flight experiments.

The primary objective during this time period was to further test and demonstrate the feasibility of obtaining EMG activity, muscle tendon recordings and video recordings during ground-based tests when the Rhesus is performing selected motor tasks. The motor tasks to be studied included the following: 1) pressing a foot pedal in response to stimuli presented by the PTS; 2) stepping on a treadmill; and 3) reaching at several postural positions.

We have been working with both the NASA Ames Research Center and Dr. Rhumbaugh's groups in these efforts. During this 5 month period, we have successfully implanted two Rhesus with seven EMG recording electrode pairs (soleus, medial gastrocnemius, tibialis anterior, vastus lateralis, biceps brachii, triceps brachii and splenius capitis) and one tendon force transducer (medial gastrocnemius). The feasibility of using three "large" cables for the exit of the wires (as opposed to the single wire exits used previously for the EMG implants) in the back region is being tested in these two monkeys. After ~2 months, the exit sites appear to be very good. These monkeys have been trained for the first two motor tasks (pressing a foot pedal and treadmill stepping) described above at Ames and we are now awaiting permission to record from them. We have made significant progress in the data management for the proposed 24-hr recordings of EMG from the implanted muscles. We have modified the DAT analysis software to generate all required data files in a single pass, reducing the time required to accomplish this tedious task. We are currently working on transferring the data display and analysis software from the Amiga to the PC and to have software available on the PC to directly view the raw EMG files during 24-hour recordings.

All of the methods and procedures used are the same as those described previously for the COSMOS flights in which we performed the same implants as is being proposed here. The new procedures being incorporated into our planned experiments are as follows. The principal procedures that are new are behavioral. To perform these studies, we have been working in collaboration with the Ames research team and with Dr. Rhumbaugh's research team in Atlanta. Rhesus have been trained to stand and step on a treadmill belt moving at speeds ranging from about 0.2 to 2.0 m/s. Our plan is to also train each Rhesus to stand in a stationary position with the upper body in varying upright positions and reach for an object. Training the animals to perform these tasks has proceeded rapidly since these motor tasks are routine for the animals. The most unique task is the execution of the foot pedal paradigms. The immediate plan is to condition the Rhesus to operate the equivalent of the joystick of the PTS, i.e., the foot pedal will be interfaced with the PTS program. In the first stage of this effort, Rhesus monkeys will be sent to Dr. Rhumbaugh's research center to begin shaping animals to operate a joystick with the foot. An additional behavior which could be benefited by the PTS system would be the reaching task. For example, if a joystick could be positioned at one of several heights, examination of EMG of muscles in the leg and force patterns of the medial gastrocnemius could be made during a well-controlled posture-reaching motor task. We would like to incorporate the PTS used with the foot task to be applied to the postural control task. The essential new element that would be required to accomplish this would be to place the PTS System at the front end and within the treadmill cover. The task itself is already one that must be performed to operate the PTS, i.e., to reach for the joystick.

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 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 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 have 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 excepted 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.

Publications, Presentations, and Other Accomplishments:

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Talmadge, R.J., Roy, R.R., Bodine-Fowler, S.C., Pierotti, D.J., and Edgerton, V.R. "Adaptations in myosin heavy chain profile in chronically unloaded muscles." Basic Appl. Myol., vol. 5, no. 2, 114-124, 1995.

Tseng, B.S., Kasper, C.E., and Edgerton, V.R. "Cytoplasm to myonucleus ratios and succinate dehydrogenase activities in adult rat slow and fast muscle fibers." Cell Tiss. Res., vol. 275, 39-49, 1994.

Zhou, M.-Y., Klitgaard, H., Saltin, B., Roy, R.R., Edgerton, V.R., and Gollnick, P.D. "Myosin heavy chain isoforms of human muscle after short-term spaceflight." J. Appl. Physiol., vol. 78, no. 5, 1740-1744, 1995.

Effect of Weightlessness on Single Muscle Fiber Function in Rhesus Monkeys

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 2/95

FY 1995 Funding: $120,000

Solicitation: NRA 88-OSSA-8

Expiration: 1/96

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion 11, 12, (9/96, 7/97)

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 (g) 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 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_o) 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_o), V_o, 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 change 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 the past year, we modified our micro-biochemistry techniques so that the assays could be conducted under oil allowing us to sample a volume as small as 5ml. As a consequence we were able to increase the number of assays obtained from a given single fiber from 2 to 12. This provides two advantages: 1. We will be able to obtain more information with smaller amounts of tissue, and 2. We will be able to detect weightlessness induced enzyme and substrate shifts not only within a population of fibers but within a given single fiber. In the next few months, we will utilize these techniques to develop a baseline data base for the slow- and fast-twitch fiber types of the Rhesus soleus and gastrocnemius muscles.

The time period between the pre- and post-flight biopsy is scheduled to be 90 days. One concern was that animal growth during this period might in itself cause significant alterations in the contractile and/or biochemical properties of the individual cells. Consequently, we characterized the contractile properties of fibers isolated from biopsies obtained from the soleus and gastrocnemius muscles of young Rhesus monkeys. To determine whether or not growth significantly altered fiber function all monkeys were biopsied twice, 4 months apart. The results showed that growth had no effect on peak force per cross-sectional area (kN/m^2) or the maximal shortening velocity (V_o, fiber lengths/s) in either the slow- or fast-twitch fibers. However, the diameter of the slow type I fiber of the gastrocnemius showed a significant increase from 51.9 to 57.8 mm. A decrease in the force per cross-sectional area was observed for the slow fiber type of both the soleus and the gastrocnemius muscle. This change appears to be at least in part due to a higher than normal value for the fibers from the first biopsy. Peak force is usually between 130 and 160 kN/m^2, while the slow fibers from the first biopsy averaged 179 and 188 kN/m^2 for the soleus and gastrocnemius muscles, respectively. The fact that growth did have some effects accents the importance of control studies designed specifically to ascertain the extent of the growth effects in the flight candidates. Since we expect weightlessness to reduce the size of the slow type I fiber, we will need to correct for the growth effect on fiber diameter.

In this contract year, we also completed data analysis and statistical evaluation of the ESDOP sit tests. Currently, we are writing 4 manuscripts that will be submitted as a final report to NASA Ames, and submitted for publication in a peer reviewed journal.

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 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 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 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.

Publications, Presentations, and Other Accomplishments:

Fitts, R.H., J.J.Widrick, and J.G. Romatowski "Effect of limb immobilization on single muscle fiber contractile function." FASED J. 8: A10, 1994.

J.J. Widrick, J.G. Romantowski, M. Karhanek, and R.H. Fitts "Contractile properties of rat, rhesus monkey, and human type I muscle fibers." Am J. Physiol, (in review).

V.P. Grishko, G. Gettleman, K. Kidd, and R.H. Fitts "Mestabolite profile of rhesus skeletal muscle fibers: effect of 16 day ESOP constraint." Med. Sci. Sports & Exerc.27: S182, 1995.

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Homeostatic and Circadian Responses of Rhesus Monkeys During Space Flight

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Funding:

Project Identification:

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Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

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.

FY 95 task progress has been concentrated in the areas of flight preparation. These have included our participation in meetings and discussions concerning flight and ground-based experimentation, generation of supporting documents, development of sensors and analysis of a ground-based study on the effect of restraint.

During an IWG meeting in Sunnyvale, we refined the four individual Discipline Experiment Management Plans (EMPs) that were drafted at the February Rhesus meeting in Paris. There is a separate Discipline EMP for Thermoregulation, Circadian Rhythms, Metabolism and Sleep. In addition, we drafted the Regulatory Team Integrated EMP (IEMP) which attempts to collate all investigations included under the category of Regulatory Biology. We also participated in the writing

and editing of the flight IEMP with our French and Russian colleagues. During a discipline IWG meeting in Paris, agreements were reached on several fronts between investigators on the Regulatory Team. We further refined the Discipline EMPs for Thermoregulation, Circadian Rhythms, Metabolism and Sleep. In addition, we viewed a demonstration of metabolism hardware and software proposed for the ground-based control studies. Numerous formal and informal meetings at NASA Ames Research Center (ARC) were held to serve the purpose of moving forward preparations for flight and ground-control studies, including equipment verification tests. We presented the Regulatory Team science objectives and integration plan to the Bion Program Review Panel.

We have actively participated in the writing and editing of the Experiment Management Plan (EMP). This has included both the Regulatory Discipline EMPs (Thermoregulation, Circadian Rhythms, Metabolism and Sleep) and the Integrated EMP (IEMP) with our French and Russian colleagues. We also drafted the Regulatory Team IEMP which includes all investigations by Regulatory Team members. In addition, we developed and presented the Regulatory Team science objectives to the Bion Program Review Panel. Our work in Experimental Design and Sensor Technology has focused on the brain and deep body temperature sensors. We have made design modifications to our brain temperature sensor. We have performed our initial surgeries evaluating the deep body temperature sensor location and probe design. To date, these studies have been performed at ARC.

Adult Rhesus Restrain Test Data included urine volumes, urine collections and DAT tapes containing electronic data consisting of body temperature and heart rate. We catalogued the urine samples, made a determination of the samples that were of the most interest and had them analyzed for melatonin content. As expected, melatonin content of the urine was highest during the first collection of the day and during the night and relatively low during the day. There were no significant differences between vivarium and restrained animals, nor were there any differences between pre and restraint time periods. Urinary volume was highest during the first collection of the day, as had been seen in other studies. There were no significant differences between vivarium and restrained animals, nor were there any differences between pre and restraint time periods.

We obtained all the software and hardware that were needed for reading the data on the tapes. Our programmer extracted this data, which was then analyzed using our laboratory data analysis programs. Body temperature rhythms showed the normal diurnal pattern usual for this species. The rhythms maintained a normal phase relationship with the light-dark cycle and did not show any consistent alterations over the period of restraint. The heart rate rhythm also had a normal, diurnal pattern throughout the experiment.

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: 1) sleep, 2) respiration, 3) endocrine, and 4) 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 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 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 rapid eye movement (REM) sleep decreased. However, the recent Spacelab 1 findings of increased rapid eye movement 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 their 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.

Publications, Presentations, and Other Accomplishments:

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Hoban-Higgins, T. M., G. T. Wassmer, D. M. Murakami and C. A. Fuller "Gravity and the circadian timing system" (Presented at World Conference on Chronobiology and Chronotherapeutics). Biol. Rhythm Res., 26(4), 401 (1995).

Murakami, D. M., T. M. Hoban-Higgins and C. A. Fuller "The effect of hypergravity on the circadian timing system" (Presented at World Conference on Chronobiology and Chronotherapeutics). Biol. Rhythm Res., 26(4), 425 (1995).

Morphological, Histochemical, Immunocytochemical, and Biochemical Investigations of Spaceflight-Related Nerve and Muscle Breakdown

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Funding:

Project Identification:

Initial Funding Date: 1/90

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Solicitation: NRA 88-OSSA-8

Expiration: 12/96

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

Responsible NASA Center: Ames Research Center

Task Description:

The results of this study will provide a better understanding of the basic cellular changes induced in primate skeletal muscles following space flight and return to terrestrial gravity. This information will benefit the design of inflight countermeasures to prevent muscle atrophy and postflight procedures for readaptation to gravity environments without muscle damage. Humans confined to chronic bed rest by illness will also benefit because the inflight procedures could be used to minimize deconditioning by maintaining muscle strength, resistance to fatigue, and coordination. The readaptation strategies will be helpful for patients reambulating following bed rest to avoid muscle reloading damage. Studies of the process of increased susceptibility to injury will also aid sports medicine prevention of muscle injuries common to movements involving unaccustomed loading.

Biopsy and electromyographic (EMG) electrode implantation procedures, which do not impede normal head and neck movements, have been defined for splenius. ESOP video has demonstrated normal head movements in restrained monkeys. Validation of light (histochemistry, immunohistochemistry) and electron microscopy (ultrastructural morphology) techniques have been accomplished for splenius tissue samples. Autoantibodies have been detected by immunohistochemistry in plasma samples from monkeys following muscle injury.

The limited capacities of the Bion capsule for data processing and storage severely impact the ability to perform EMG and video for this experiment. Priorities for utilizing blood limit the availability of plasma for autoantibody analysis. These payload constraints necessitate reducing the scope of this experiment to the light and electron microscopic studies of the cellular changes in splenius undergoing space flight-induced muscle atrophy and damage.

Humans returning to Earth after 1-2 weeks of space flight experience delayed-onset soreness, fatigue, faulty coordination and weakness of antigravity skeletal muscles indicating pathological muscle damage. These deficits may compromise human performance and safety when transitioning between microgravity and terrestrial gravity. Our studies of space flown rats (SL-3, Cosmos 1887 & 2044, SLS-1, SLS-2) have demonstrated that atrophic muscles show elevated susceptibility to injury during postflight reloading resulting in pathological destruction of muscle fibers. The proposed rhesus monkey Bion studies will define the cellular and biochemical basis for space flight-induced muscle weakness in a space flown primate whose muscles are closer to human muscles in size, structure, biochemistry and rate of adaptation. The splenius captius neck muscle was selected for study because it holds and moves the head against gravity, and in contrast to lower limb muscles, normal function continues when the monkey is restrained in the Bion chair. The Bion constraints of limiting upper and lower limb movements caused this investigator to shift from studying the soleus and deltoid muscles to the splenius in order to test of the effects of microgravity unloading. Microgravity unloading is expected to produce splenius muscle atrophy, and reentry load stresses on the head are anticipated to induce muscle damage. The rhesus preparation models atrophy of human neck (back) muscles which are vulnerable to injury by reentry stresses on the head supporting the added burden of a space helmet.

Splenius contains a mixture of fast and slow muscle fibers which permits assessment of atrophy and damage on muscle fiber types. Microgravity is expected to produce atrophy and increased fast myosin expression in slow fibers assayed histochemically and immunohistochemically. Splenius contractile activity, as monitored by EMG, will indicate fewer muscle contractions and increased fatiguability. Inflight video will show that normal head and neck movements occur during space flight. Reloading will cause slow fiber destruction and interstitial edema leading to muscle tissue death analyzed by electron microscopy. Immunohistochemical staining will reveal that autoantibodies are generated against leaked muscle cell components and potentially exacerbating cell damage.

Publications, Presentations, and Other Accomplishments:

Anders, S. "Effects of muscle injury and restraint-induced atrophy on circulating antibody levels." Wisconsin Space Grant Consortium Annual Meeting, 1994.

Behavior and Performance Project

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Funding:

Project Identification:

Solicitation: NRA 88-OSSA-8

Initial Funding Date: 3/95

Expiration: 1/96

FY 1995 Funding: $200,010

Students Funded Under Research: 5

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

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).

Scientists in the Behavior and Performance Project have adapted the goals and experiments from the Rhesus Project in accordance with opportunities and limitations of Bion 11. We demonstrated that juvenile rhesus monkeys could be trained to respond to computerized tasks by manipulating a joystick with either a hand or a foot. This finding has practical implications for integration of Behavior and Performance Project goals with those of the Russian investigators.

We established procedures for training monkeys in Moscow to perform the PTS tasks. We continue to support this training both by travelling to Moscow and by analyzing all training data.

Numerous support studies have been conducted in support of our science. We demonstrated the rhesus monkeys could quickly be trained to walk on a treadmill for studies of locomotion. The effects of one-hour versus continuous access to the PTS were also examined. We conducted experiments on the effectiveness of the PTS as an enrichment device, and on continuities between humans' and monkeys' memory and attention. We also analyzed and reported the Adult Rhesus Restraint Test data, which demonstrated that the psychological measures we collect are sensitive to effects of restraint and physiological procedures.

These activities provide the basis for our involvement in Bion 11. The research continues to validate the rhesus monkey as an effective model for many aspects of human behavior, and suggests the importance of developing flight hardware for future Psychomotor Test System experiments. Finally, effective support of the psychological well-being of the research animals is absolutely critical for the ongoing success of NASA's life sciences research.

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.

Publications, Presentations, and Other Accomplishments:

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Washburn, D.A. "Teaching of Psychology (Contribution: What Monkeys Can Do)." (in press).

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Washburn, D.A. "A Comparative Investigation of Working Memory: Rehearsal in the Sketchpad?" Meeting of the Southern Society for Philosophy and Psychology, Virginia Beach, VA, April 1995.

Washburn, D.A. "What Animal Cognition Tells Us about Human Cognition." Conference of the Cognitive Science Society, Atlanta, GA, August, 1994.

Washburn, D.A. "Monkeys in Outer Space." Fernbank Museum of Natural History Summer Lecture Series, Atlanta, GA, August, 1994.

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Bone and Lean Body Mass Changes Following Space Flight

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Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1995 Funding: $62,000

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Students Funded Under Research: 0

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: None

Task Description:

This proposal has been integrated with the proposal by Dr. Zerath entitled, "Bone Tissue and Cell Effects of Spaceflight on young Rhesus monkeys."

Techniques to position and reproducibly scan the rhesus monkey were developed. To document repositioning error, six monkeys were scanned before and after repositioning. For this purpose the complete scanning protocol was performed after which the animal was moved, repositioned and re-scanned. The results of this testing demonstrated excellent short term reproducibility.

Reproducibility of the techniques over a 20 day test period was also investigated. For this purpose six animals were scanned at a 20 day interval and the percent standard deviation was calculated and a power analysis performed. The regional analysis data showed that decreases in muscle mass on the order of that documented to occur in humans during an 8 day shuttle flight would be detectable in these animals. Also bone atrophy of about 2-5% would also be detectable.

An 18 day immobilization test was completed in which three experimental monkeys and two controls were scanned before and after the immobilization period. This data did not show significant bone or lean tissue changes.

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 one 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 U.S. 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.

Publications, Presentations, and Other Accomplishments:

Zerath, E., V. Novikov, A. Leblanc, A. Bakulin, V. Oganov, and M. Grynpas. Effects of Space flight on Bone Mineralization in the Rhesus Monkey. J. Appl. Phys., (in press).

Immunology Spaceflight and Immune Responses of Rhesus Monkeys

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Funding:

Project Identification:

Initial Funding Date: 10/90

FY 1995 Funding: $57,000

Solicitation: NRA 88-OSSA-8

Expiration: 10/99

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

Responsible NASA Center: Ames Research Center

Task Description:

Evidence from both human and rodent studies has indicated that alterations in immunological parameters occur after space flight. The number of flight experiments has been small, and the full breadth of immunological alterations occurring after space flight remains to be established. Among the major effects on immune responses after space flight that have been reported are alterations in lymphocyte blastogenesis and natural killer cell activity, alterations in production of cytokines, changes in leukocyte sub-population distribution, and decreases in the ability of bone marrow cells to respond to colony stimulating factors. Changes have been reported in immunological parameters of both humans and rodents. The significance of these alterations in relation to resistance to infection remains to be established. The objective of the studies contained in this project is to determine the effects of space flight on immune responses of rhesus monkeys. The hypothesis is that space flight and the attendant period of microgravity will result in alteration of immunological parameters. The parameters to be tested include production of cytokines, composition of leukocyte subpopulations, functional activities of immunologically significant cells, and differences in effects on cells from primary and secondary lymphoid tissues. The expected significance of the work is a determination of the range of immunological functions of the rhesus monkey, a primate similar in many ways to man, affected by space flight. Changes in immune responses that could yield alterations in resistance to infection may be determined. The duration of alterations in immune responses may also be determined. This could yield useful information for planning studies that could contribute to crew health. Additional information on the nature of cellular interactions for the generation of immune responses may also be obtained.

In the past fiscal year, we have continued our transition to the Bion platform and development of assays for immunological parameters of rhesus monkeys. We have been able to show that anti-human monoclonal antibodies could be used to identify rhesus monkey interleukins-1 and -2 as well as the interleukin-2 receptor. We also developed techniques for the induction of the interleukins I rhesus monkeys. We have also been working on the development of polymerase chain reaction techniques for detection of message for rhesus monkeys cytokines. Additionally, we completed and analyzed the data from the Adult Rhesus Restraint Test. This test examined the effects of restraint under conditions of gravity. Four experimental monkeys were restrained for 17 days, while 2 control monkeys were monitored in vivarium conditions. Blood samples were collected at 4 time points prior to restraint (-76, -41, -10 and -4 days) and two points post release (+17 and +24 days). After initiation of restraint, an experimental animal was removed from the study due to procedural difficulties. The samples were analyzed for production of interferon-alpha (IFN-a) and interferon-gamma (IFN-g) in response to mitogen stimulation. The samples were then stained with various fluorescent antibodies for neutrophils (CD16), T cells (CD3), CD4+ and CD8+ T cells, B cells (IgM and IgG), and for major histocompatability complex class II antigen (HLA-DR), and analyzed by flow cytometry. Students' T-tests and Paired Students' T-tests were used to analyze data. Prior to restraint, slightly elevated levels of IgM ($p<0.05$) and CD8+ T cells ($p<0.05$) were observed in the experimental group. A significant decrease in CD8+ T cells ($p<0.05$) was then observed in the restraint group between days -4 and +17. No other differences were observed post restraint between control and experimental groups for all parameters measured ($p>0.05$). Interestingly, levels of neutrophils, CD8+ T cells, and Hu-IgM staining of B cells sharply declined from day -10 to day -4 ($p<0.05$). Change of environment and physical examinations could have played a role in the decrease observed between -10 and -4 days. These manipulations should be examined so that physical effects just prior to restraint can be minimized. Examination of data post restraint reveals that restraint does not play a major role in the effects of space flight on the immune parameters, and should not influence greatly the results of our Bion flight experiment.

The proposed Bion experiments are designed to demonstrate if the rhesus monkey will be useful as a surrogate for humans to determine the effects of space flight on immune responses. We hope to be able to determine effects of space flight on a broad inclusive range of immunologic parameters of rhesus monkeys. We will also be able to see if there are differences between local and systemic effects of space flight on immune responses. New immunological and molecular biology techniques will be applied to determine effects of space flight on immunologic parameters not previously examined. When we are successful in establishing the model, it could be used in the future to answer questions that both our previous studies as well as a joint NASA/National Institute of Allergy and Infectious Disease panel of which Dr. Sonnenfeld was a member indicated were important questions for the future. These include whether space flight actually affects the ability to immunize, resistance to infection, and resistance to tumors. These data could be useful in furthering the use of the rhesus monkey as a model for human diseases of a similar nature on the ground. New therapies could be developed using the rhesus monkey as such a model.

Publications, Presentations, and Other Accomplishments:

Morton, D.S., J.P. Swiggett, A.M. Hakenewerth, N.A. Fowler and G. Sonnenfeld "Effects of movement limitation on immunological parameters. " American Society for Gravitational and Space Biology, Bulletin 9, no 31, 1995.

Sonnenfeld, G., D.S. Morton, J.P. Swiggett, A.M. Hakenewerth and N. Fowler "Movement Lifitation and immune responses of rhesus monkeys." NASA Tech Brief, (Submitted for NASA Technical Memorandum), 1995.

Sonnenfeld, G., L. Schaffar, D.A. Schmitt, C. Peres and E.S. Miller "The rhesus monkey as a model for testing the immunological effects of space flight." Adv. Space Res., vol 14, 395-397, 1994.

Adaptation to Microgravity of Oculomotor Reflexes

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Funding:

Project Identification:

Solicitation: NRA 88-OSSA-8

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Expiration: 9/95

FY 1995 Funding: $150,000

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Bion 11, 12 (9/96, 7/97)

Responsible NASA Center: Ames Research Center

Task Description:

In space, the otoliths constantly sense only a fraction of gravitational force and momentarily receive only small amplitude linear accelerations during head translations. Thus, it might be expected that otolith-ocular reflexes that are mediated by the linear vestibulo-ocular reflex (LVOR), such as ocular counter-rolling (OCR) and ocular vergence that orient the eyes to gravity, would be depressed after adaptation to microgravity. In accord with this, the amplitude of two otolith-ocular reflexes, OCR and ocular vergence, were reduced for 11 days after the COSMOS 2229 space flight in two flight monkeys. The LVOR induced by sinusoidal linear acceleration on a sled along interaural (IA), naso-occipital (NO) and dorso-ventral (DV) axes was also reduced in one of the two flight monkeys. Thus, otolith-ocular reflexes that orient the eyes to gravito-inertial acceleration (GIA) were changed over relatively long periods of time after reentry.

The purpose of this research is to study how spatial orientation of the linear and angular vestibulo-ocular reflexes (LVOR and AVOR) of monkeys are altered by space flight. We will use eye movements produced by or dependent on the otolith organs and the semicircular canals as measures of this orientation. Changes in the AVOR during active gaze shifts that involve head and eye movements will be recorded in space by the Russians and compared to the AVOR recorded during passive rotation on Earth. Finally, we will use binocular three-dimensional recordings in our ground-based recordings to enhance our understanding of how eye movements are affected by changes in the GIA before and after space flight. We postulate that otolith-induced or dependent eye orienting responses that tend to align the eyes to gravito-inertial acceleration (GIA), will either be reduced after space flight, as for OCR and vergence or as for velocity storage, or will be shifted to align with a body axis. Active gaze shifts that

involve head movements in space will be altered, but there will be no changes in the passive AVOR recorded on earth.

For the Bion 11 flight, laboratories will be set up in Moscow (Spring 1996) to test a control group of 12 rhesus monkeys. We will use sinusoidal linear acceleration on a sled. During the summer, two of the animals from the group of 12 will fly in the Bion space capsule for approximately two weeks. While in space, the monkeys will perform behavioral tasks, moving their head and eyes toward lateral visual targets and will press a light with their hands to receive a juice reward. While in space, the monkeys will be tested for spontaneous nystagmus using electro-oculography (EOG). At the end of the flight, the capsule will be returned to Earth and the animals will be recovered and returned to Moscow for testing. Postflight testing will extend for 14 days at the Institute of Biomedical Problems. All ground-based testing will be done with binocular three-dimensional eye coil recordings. It is hypothesized that otolith ocular reflexes such as OCR, horizontal and vertical LVOR and ocular vergence, will be reduced for 5-7 days after space flight.

The principal investigator successfully completed analysis of data collected during the Rhesus ARRT experiment. Results provide a detailed description of the animals' heart rate/variability adaptation to long duration physical restraint. The main finding was that heart rates initially fell, reflecting adaptation, but by restraint day 5 they increased and did not return to baseline for the remaining 16 days of restraint. Results suggest that long-duration restraint may affect measurement of other variables. Three manuscripts were prepared to report the results to the Ames Project Office to fulfill the final report requirements of the Rhesus Project's SIT and ARRT tests. They are listed on the bibliography page. These papers have been sent out for preliminary review in preparation for submission as NASA TMs, and then will be prepared for submission to an appropriate journal. Analysis of Cosmos 2229 results was conducted. Linear vestibulo-ocular reflexes (LVORs) provide inertial stability for vision during linear head motion. Bilateral search coils in 12 rhesus monkeys were used to characterize LVORs before and after an 11 day space flight by 2 flight animals and controls. Linear motion was delivered along inter-aural (IA), naso-occipital (NO), dorso-ventral (DV) and intermediate, oblique head axes. Response gain and phase were calculated, along with gaze position and vergence state. The gain and phase of differentiated, de-saccaded eye position recordings were calculated using Fourier analysis. Preflight LVORs were similar to previous results in squirrel monkeys. LVORs compensatory for head displacement were recorded during IA, DV, NO, and intermediate axis motion. All responses were affected by vergence state (visual target distance). Post-flight, during IA motion (5 Hz,0.5g), M906 showed a roughly 2/3 reduction in the slope of the function relating horizontal LVOR sensitivity to vergence that had not recovered by R+391 hours. Under the same conditions, M151 showed almost identical responses pre- and post-flight. During DV motion (5 Hz, 0.5g), M906 showed from 35-60% reduction in the slope of the function relating vertical LVOR sensitivity to vergence that had not recovered by R+391 hours. Under the same conditions, M151 showed responses immediately post-flight that were almost identical to preflight values, and subsequently had reductions of 30-50%. During IA and DV head motion at 5 Hz (0.5 g), M906 had large reductions in the slope of the function relating LVOR sensitivity to vergence that did not recover by R+391 hours. Under the same conditions, M151 showed almost similar responses pre- and post-flight.

Studies like these are necessary if there is to be continuing manned space flight. With a continuing presence of the US in space for strategic or other purposes, it will be essential to support human personnel with appropriate research on changes they will encounter after adaptation to microgravity.

The ability to understand how balance and coordination are affected by space flight should prove of great value in understanding imbalance in the elderly. The ability to understand how balance and coordination are affected by space flight should prove of great value in understanding postural and locomotion difficulties encountered by people with diseases or strokes that affect parts of the brain that process information related to spatial orientation (e.g., basal ganglia - Parkinson's Disease, cerebellum, brainstem).

It is currently thought that some physiological changes that occur during space flight might be good analogues to terrestrial changes that occur during aging and disease, and therefore that findings from space experiments might be helpful to sick and aging humans on Earth. During aging and while experiencing the microgravity environment of space, sensorimotor function may be similarly challenged: changes and ambiguities in sensory inputs lead to potential errors in cognition and perception affecting equilibrium and spatial orientation. Errors in reflexes and perceptions can lead to dysfunctional consequences, such as falls in the elderly and decrements in motor control in astronauts.

Effect of Microgravity on Osteoblast Gene Expression

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Initial Funding Date:

FY 1995 Funding: $

Solicitation: 94 OLMSA-03

Expiration:

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Biorack, S/MM-06, STS-84, 6/97

Responsible NASA Center: Ames Research Center

Task Description:

This flight proposal aims to analyze how microgravity effects bone loss by investigating alterations in osteoblast gene expression patterns in microgravity. We will look at key genes responsible for osteoblast growth activation and cell cycle regulation. These include the gene expression patterns of prostaglandin E2 (PGE2) synthesis and actin; c-PLA2 (cytosolic phospholipase A2, COX-1 and COX-2 (cyclo-oxygenases), and the PGE2 receptors EP1, EP2, and EP3 and growth biomarkers c-fos, PCNA and statin. 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. Mechanical stress used as a countermeasure for bone loss has been demonstrated to cause release of PGE2 from osteoblasts. PGE2 increases trabecular bone formation in rats. Studies from this laboratory have show that PGE2 causes activation of c-fos from 15 to 60 minutes followed by osteoblast growth at 24 hours. The lack of PGE2 synthesis occurring in space may be a critical factor responsible for the bone loss that occurs in astronauts. PGE2 down regulation is likely a key component in the mechanism ob bone loss that occurs in astronauts.

Since only 5-10 ug of total RNA are expected from the Type I cell chambers, we have perfected the rtPCR method for analysis of expression. We have tested our cells in flight on CMIX hardware.

We have been selected for definition for the first Biorack experiments and will be preflight testing Biorack Type I containers. Using rtPCR technology, expression patterns will be analyzed in osteoblasts activated and fixed while exposed to 0.0, 0.3, 0.6, and 1.0-gravity; cell cell growth, PGE2 synthesis and glucose will also be analyzed. No additional data was provided by the investigator for this research.

Microgravity Effects on Bone Cell Gene Expression

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-07

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Biorack, S/MM-03

Responsible NASA Center: Ames Research Center

Task Description:

This flight proposal aims to analyze how microgravity effects bone loss by investigating alterations in select gene expression patterns. We will look at key genes responsible for osteoblast growth and homeostasis. These include the gene expression patterns of the elements responsible for prostaglandin (PG) E2 synthesis and action; c-PLA2 (cytosolic phospholipase A2), COX-1 and COX-2 (cyclo-oxygenases), and the PGE2 receptors EP1, EP2, and EP3. Additionally, we will analyze the expression pattern of the gene coding for the stress protein, HSP70, involved in maintaining cellular homeostasis. Expression patterns will be analyzed in osteoblasts exposed to microgravity using rtPCR technology. 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. Mechanical stress used as a countermeasure for bone loss has been demonstrated to cause release of 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 down regulation is likely a key component in the mechanism of bone loss that occurs in astronauts. Analysis of HSP70 may indicate the level of stress the osteoblast is experiencing.

Osteoblasts will be growth activated upon exposure to the microgravity environment. Cells will be flown in the Tissue Culture Incubator located in the orbiter middeck. Growth activating the cells in space will eliminate any stresses induced during the launch phase. Prior to mission completion the cells will be fixed in guanidinium isothiocyanate. This fixation will preserve any mRNA species expressed. Fixing the cells while still in the microgravity environment will eliminate any new changes in gene expression that may occur during the orbiter's return to Earth. Total RNA will be

prepared from flight and control cells using standard procedures. DNA copies of RNA species will be produced using reverse transcriptase and random hexamer primers. The DNA copies are more stable to degradation than RNA. Using PCR primers specific for the genes mentioned above, we will amplify any expressed mRNA transcripts and subsequently analyze them by gel electrophoresis and densitometry.

No additional data was provided by the investigator for this research.

Graviperception in Starch Deficient Plants in Biorack

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 94 OLMSA-03

Initial Funding Date: 9/95

Expiration: 8/96

FY 1995 Funding: $77,606

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Biorack ,S/MM-05, STS-81, 12/96, & -06, STS-84, 5/97

Responsible NASA Center: Ames Research Center

Task Description:

The purpose of the proposed 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. The proposed research is for two flight missions. 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 first year of the proposed program will focus on biocompatibility testing of the "lentil-roots" hardware (LRH) with *Arabidopsis* WT and mutant seedlings and ground-based studies of integrin distribution in *Arabidopsis* roots.

We are in the definition and development phase for space flight experiments on the ESA Biorack payload on S/MM-05 (STS 81) and S/MM-06 (STS 84). Our accomplishments to date include: 1) The internal configuration of the Lentil-Roots minicontainers has been successfully modified for growth of *Arabidopsis* seedlings. We use a series of filter papers and cellulose nitrate membranes to grow the seedlings. This ESA-supplied hardware previously was used for much larger lentil seedlings; 2) The

light pretreatment for maximal germination of *Arabidopsis* seeds has been determined to be 14 hours. Maximal germination is highly desirable for flight experiments; and 3) The Cannon L1 video camera can be used for our experiments on S/MM-05. We went to Hanger L at the NASA Kennedy Space Center to test the video camera in the ground Biorack unit. It has sufficient resolution to successfully image the small *Arabidopsis* 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.

Publications, Presentations, and Other Accomplishments:

Katembe, W.J., C.A. Makaroff, J.Z. Kiss "Identification of integrins in plant cells (abstract)," American Society of Plant Physiologists, Charlotte, NC. Plant Physiology, 108(S), 92 (1995).

Kiss, J.Z. "The response to gravity is correlated with the number of statoliths in *Chara* rhizoids." Plant Physiology, 105, 937-940 (1994).

Kiss, J.Z., M.M. Guisinger, A.J. Miller, J.B. Wright "The response to gravity is correlated to the amount of starch in *Arabidopsis* intermediate-starch mutants (abstract)." American Society for Gravitational and Space Biology, Bulletin 9, 38 (1995).

Kiss, J.Z., T. Caspar, J.B. Wright, A.J. Miller "Gravitropism in roots of intermediate-starch mutants of *Arabidopsis* (Abstract)", American Society of Plant Physiologists, Charlotte, NC. Plant Physiology, 108(S), 24 (1995).

Wang-Cahill, F., J.Z. Kiss "The statolith compartment in *Chara* rhizoids contains carbohydrate and protein." American Journal of Botany, 82, 220-229 (1995).

Mechanisms of Gravity Sensing and Response in Hematopoietic Cells

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Funding:

Project Identification:

Solicitation: 93 OLMSA-07

Initial Funding Date: 1/95

Expiration: 5/97

FY 1995 Funding: $125,000

Students Funded Under Research: 2

Joint Participation: ESA

Flight Information:

Flight Assignment: Biorack, S/MM-03, STS-76, 3/96

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Biorack Cytokines H/W

Task Description:

The overall objective of this proposal is to investigate the role of the cytoskeleton in gravity (microgravity) "sensing" and signal transduction in single cells (lymphocytes). Specific aims are 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 results show altered actin morphology in mouse and Xenopus cells flown on STS-52 and 56 and HL 60 cells flown on STS67 and STS-69. The effect of altered cytoskeletal morphology on signal transduction is unknown and will be investigated by probing for gene products by RT-PCR after growth stimulating cells in microgravity. The proteins gelsolin and proftlin regulate actin polymerization and filament formation in cardiac myocytea. 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. The role of antigen presenting accessory cells is controversial. Although an objective of the original proposal, the research to evaluate dendritic/T cell interaction during lymphocyte activation will be conducted on a different payload. Instead, the S/MM-03 research will concentrate on the RT-PCR evaluation of mRNA for a number of genes and the morphological alterations of the cytoskeleton. Signal transduction evaluation will include visualization of translocation of protein kinase C (PKC) from cytosolic stores to membrane sites after stimulation of Jurkat cells with serum, rather than with specific mitogens, in microgravity .

First year activities to verify procedures and expand ground-based data are 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; 3)

conduct an Experiment Sequence Test at NASA KSC to ensure cell survival and appropriateness of all procedures. Second year activities include flying the first experiment to evaluate these parameters in microgravity.

The most significant change in direction of this research is that, on this one flight opportunity, I cannot fly the primary human T lymphocytes exposed to Con A and anti-CD3 as I originally proposed. Hopefully some of the original objectives using T cells can still be met through my collaboration (which was formalized in FY95) with Co-investigator, Dr. Didier Schtnitt. I have omitted testing dendritic and T cell interaction and will plan this for a different payload.

As stated in the Task Abstract, the objectives for the first year included verifying procedures and expanding ground-based data, developing the prisners to evaluate mRNA gene transcription products by RT-PCR, testing cytoskeletosl/protein kinase C (PKC) interactions by immunofluorescence, familiarization with the Biorack hardware and conducting the Experiment Sequence Test (EST) at KSC. All of these tasks were accomplished. Specific achievements for the first year involved set up of the project and included hiring a molecular biologist and a tissue culture technician, and purchasing a Genequant, a thermocyder, and a gel electrophoresis system for the RTPCR assays. Scientific achievements included verification of techniques for RNA extraction, cytoskeletal morphology visualization and focalization of PKC in activated versus non-activated cells. One of the planned original activities (evaluating dendritic T cell interaction by flow cytometry) and one technique (use of western blotting to evaluate early signal transduction events) were dropped from the study because we chose to fly Jurkat cells rather than primary human lymphocytes. Jurkat cells were chosen for the SIMM-03 opportunity as a systematic approach using a fail-safe model cell type in lieu of the less reactively stable primary lymphocytes. I also chose to fly six replicate wells of Jurkat cells per hardware unit, per time point, for each of two preservation methods, rather than to fly two cell types and risk not having adequate replicates for the new RNA evaluations key to the main objective of this flight opportunity. Our original plan was to fly primary lymphocytes on the second and third Biorack opportunities; however, our renewal proposal was not selected for further funding after S/MM-03.

During FY95, we provided documentation including the Ground Support Requirements Document, fluids lists, laboratory procedures for Hangar L activities, and other documentation developed in cooperation with the ESA and NASA/Ames mission management team. We performed hardware check out and functional verification, purchased parts including a pipetor and other materials for flight and ground-based control tests. Other accomplishments included travel to Noordwijk in December 1994 for training on the Biorack "Cytolcines" and other hardware and a one week RT-PCR training session in Dr. Millie Hughes-Fulford's laboratory to become familiar with current technology.

We have demonstrated that the Biorack hardware is an excellent choice for the TCELL experiment. Do the throat cells grow and remain viable during the 48 hour ten plus pre-launch time? Our ground-based tests showed that cell counts and viabilities for Jurkat cells held in the hardware were within the ranges existed. Can we get good replication of counts, viabilities and RNA from cells grown in the Biorack hardware? Results from the EST at KSC in October, 1995 showed good well-to-well replicability for cell counted glucose levels and RNA extraction.

Immunofluorescence microscopy procedures have been refined to show actin and tubulin cytoskeleton and nuclear morphology in cells grown in the Biorack hardware. The RNA extraction procedure details are defined and were verified on the EST cells. We have shown migration of PKC from the cytosol to the cell membrane in activated cells. Are hardware preparation procedures verified? Procedures and questions of hardware sterilization and cell handling during loading and unloading and storage have been answered. Which gene products will be evaluated? We have selected primers to evaluate by RT-PCR the following: beta actin, constitutive and inducible heat shod proteins, several cytoskeletal associated proteins, cyclophilin, c-fos and c-myc.

What additional primers will be selected for analysis? We will determine this after evaluating RNA from the EST cells by RT-PCR. What are the minimal G levels that affect cytoskeletal assembly and morphology? We continue to show on other payloads that cytoskeletal morphology in flown cells is altered. Why should low-g affect the coalescence or polymerization of the actin and tubulin filaments? How does this alteration affect the transduction of signals from membrane to nucleus and back? Which genes are affected? How are the MAP kinases involved or is phosphorylation of MAP kinase cascade affected in low-g?

This year's progress has been extremely significant considering that the lab was not staffed and no equipment for RT-PCR was in place. We have now developed a full laboratory capability to test any number of gene products from RNA extracted from flown or ground cells. This is, after all, the point of cellular control basic to all cellular function. The effect on future work on this taste is that we are now set up to perform these assays for our own research and can perform gene product assays for co-investigators and other investigation. The possibility of answering the new questions generated by this research is critical to the continuity of NASA's gain from initial funding to this project and will provide significant information from future flight and ground research.

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 in-flight 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 immunocompetence 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.

Clearly, 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 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. My colleagues and I 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 on STio7 and 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.

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

This research, directly probing gene expression and regulation in the cells involved with cellular immunity, is focused on the very basic molecular mechanisms of cytoskeleton related signal transduction and gene expression. The short term problems encountered by humans in space flight have similarities to illnesses on Earth for which no effective treatments have been developed (AIDS and some forms of cancer).

Gene therapy and control of cell growth at the gene and molecular level is already being tested clinically. The results of our research can advance understanding of basic regulatory mechanisms involved in transduction of signals in activated cells and the genes that control the processes. 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.

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.

Publications, Presentations, and Other Accomplishments:

Hughes-Fulford, M. and Lewis M.L. "Effects of Microgravity on osteoblast growth activation." Submitted and accepted experimental Cell Research, 1995.

Modification of Radiogenic Damage by Microgravity

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Initial Funding Date:

FY 1995 Funding: $

Solicitation: 93-OLMSA-07

Expiration:

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Biorack ,S/MM-03, STS-76, 3/96

Responsible NASA Center: Ames Research Center

Task Description:

The purpose 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.

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* will be used to measure forward autosomal recessive lethal mutation using a balancer chromosome technique and stable anaphase bridges in intestinal cells will be scored histologically to quantify chromosome aberration. 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.

No additional data was provided by the investigator for this research.

Bacterial Growth on Surfaces in Microgravity and on Earth

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Co-Investigators:

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Funding:

Project Identification:

Solicitation: 94 OLMSA-03

Initial Funding Date: 7/95

Expiration: 6/98

FY 1995 Funding: $73,185

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Biorack ,S/MM-05, STS-81, 12/96, Euro-Mir

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 date 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 July, the PI traveled to Toulouse, France, for familiarization with the Phorbol Type I containers selected for use with this experiment, returning with two containers on loan. Samples of the polycarbonate container material and other components were also obtained.

Biocompatibility experiments were performed to determine if the container materials or lubricant (silicone grease), had any effect on the growth of the organism *Burkholderia cepacia*. This extremely oligotrophic (low-nutrient) organism was cultured in sterile reagent grade water (Milli-Q) and incubated with the components singly and in combination. No inhibition or stimulation of growth was detected.

In early November, the PI traveled to NASA's Kennedy Space Center for briefing with ESTEC and NASA ARC staff involved in the project, and reported on the initial biocompatibility experiments.

Two undergraduate students were selected from among eight qualified applicants to assist with the project. They are both juniors majoring in microbiology at Montana State University. Carla Johnsrud is enrolled in the Medical Technology option, while Ryan Storfa is in the Environmental Health option. The PI began briefing them at the end of the Fall semester in December in preparation for their active involvement from the beginning of the Spring semester, 1996. Both are intending to continue on the project until their graduation at the end of the Spring 1997 semester.

There are no distinct earth benefits from the work done from July through December 1995, as all of the work has been ground-based and is preparatory to the proposed experiment.

Gravitropism and Autotropism in Cress Roots

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Co-Investigators:

Dietr Volkmann (Principal Investigator)

Funding:

Project Identification:

Solicitation: NRA 94-OLMSA-03

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 1

Joint Participation: ESA

Flight Information:

Flight Assignment: 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 μg, 0.1g, and 1g 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 γ-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.

Bratislav Stankovic started as a postdoc on the project on November 1, 1995. The timeline of the flight experiment has been revised, in consultation with Dieter Volkmann and European Space Agency (ESA) personnel. Procedures for ground-based controls are being developed e.g. clinostats are being assembled. A method for visualizing the cytoskeleton in fixed, embedded (in Staedtmann's wax) and sectioned cress roots has been worked out. Critical cytoskeletal antibodies have been located. An important antibody now available to us is against plant *rho* proteins, a possible organizer of F-actin. Immunofluorescence trials using this antibody are in progress. The Columbus-based team has begun to gain familiarity with Biorack and NIZEMI hardware specific to this experiment. The literature on plant autotropism has been reviewed (in house) to start to place flight data in a wider intellectual and evidential context.

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 the common man 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.

Publications, Presentations, and Other Accomplishments:

Sack, F.D. "Cell biology of plant gravity sensing. Advances Space Research", Vol 14, no 8, 117-119 (1994).

Sack, F.D., D. Kim and B. Stein "Organelle sedimentation in gravitropic roots of *Limnobium* is restricted to the elongation zone." Annals Botany, vol 74, 35-42 (1994).

Schwuchow, J.M. and F.D. Sack "Microtubules restrict plastid sedimentation in protonemata of the moss *Ceratodon*." Cell Motility and Cytoskeleton, Vol 29, 366-374 (1994).

Schwuchow, J.M., D. Kim and F.D. Sack "Caulonemal gravitropism and amyloplast sedimentation in the moss *Funaria*." Canadian Journal Botany, vol 73, 1029-1035 (1995).

Walker, L.M. and F.D. Sack "Microfilament distribution in protonemata of the moss *Ceratodon*." Protoplasma, vol 189, 229-237 (1995).

Walker, L.M. and F.D. Sack "An ultrastructural analysis of cell component distribution in apical cells of *Ceratodon protonemata*." Protoplasma, vol 189, 238-248 (1995).

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

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Funding:

Project Identification: 79 US - AGGS

Solicitation: 94 OLMSA-03

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $75,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Biorack ,S/MM-05,-06; STS-81, 12/96; STS-84, 6/97

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 ground-based, 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 1) the formation of intercellular signaling complexes, 2) cytoskeletal transitions in response to these events. Experiments testing the resulting intracellular signaling will be addressed in a separate Biorack Flight Opportunity proposal "Effects of Microgravity on Lymphocyte Activation: T Cell Receptor-Mediated Signal

Transduction and Cell Cycle Regulation". Together, these experiments will provide insights into the potential biophysical mechanisms involved in the inhibitory effects of gravity on cells and tissues.

This effort is a new start which was formally initiated in FY 96. Preliminary studies were performed in FY 95 to verify the technical feasibility of experimentation in the AGGREGATE hardware. In addition, detailed biocompatibility studies were performed using the hardware components and materials. A need for modification of the hardware was identified during this testing.

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 tents 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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Co-Investigators:

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Funding:

Project Identification:

Initial Funding Date: 9/95

FY 1995 Funding: $93,568

Solicitation: 94 OLMSA-03

Expiration: 8/96

Students Funded Under Research: 1

Flight Information:

Flight Assignment: Biorack ,S/MM-05, -06; STS-81, 12/96; STS-84, 6/97

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 as compared to Earth-normal gravity (ENG). A previous unmanned 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 1g force were found to contain higher levels of ATP. Since ATP is critical to the maintenance of sperm motility, it is likely that microgravity may produce changes in sperm motility. Sperm motility is regulated by the content of cAMP, calcium (Ca^{2+}), and the state of protein phosphorylation modulated by these second messengers. Whether these components are altered during changes in gravitational forces are not known. The present study will examine, using ESA Biorack Hardware, changes in ATP, ADP, cAMP, cGMP, and protein phosphorylation in sea urchin and mouse sperm under a variety of physiological and gravitational conditions, including those which, in ENG, promote oocyte fertilization. The research project comprises 4 specific aims: Aim 1: Determine whether ATP, ADP, cAMP, and protein phosphorylation in sperm are altered in microgravity in replicate semen samples. Aim 2: Determine whether ATP, ADP, cAMP, cGMP, and protein phosphorylation are altered in microgravity in sperm incubated under *in vitro* fertilization conditions, known to promote hyperactivated motility. Aim 3: Examine whether ATP, ADP, cAMP, cGMP, and protein phosphorylation in sperm are altered in water of different density at ENG (i.e. in D_2O) and compare to results obtained in aim 2. Aim 4: If differences in ATP, ADP, cAMP, cGMP and protein phosphorylation in sperm under the conditions described in specific aims 1, 2, and/or 3 are observed, determine whether these are correlated with changes increases in internal Ca^{2+}. 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. These studies will 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. These studies will yield information relevant to plans for habitation and survival during long-term space flight.

The plan for the first year will be 1) Prepare first set of sperm samples and set up the methods for transport and incubation of sperm samples, 2) Assure biocompatibility of Biorack hardware for planned experiments, 3) Plan fine details of the conduct of the experiments to ensure matched protocols and identical treatment of samples for both SPFE and ENG experiments, 4) perform technician and flight crew training, and 5) conduct simulations and first set of flight experiments and ground controls.

Progress towards making the proposed experiments ready for flight has been proceeding extremely well. Biocompatibility studies have been successfully completed for the sea urchin sperm that will be used for both SS/M-05 and SS/M-06. Our tests demonstrated that the sperm will survive in the Biorack H/W extremely well under launch scrub conditions as long as 72 hr with no significant deterioration in cell viability, and as long as 96 hr with only a slight reduction in viability. The initial timeline discussions indicated that the experiment will be conducted at ~30 hr ± 12 EMT which is well within the limits of optimal cell viability. The results of these experiments were presented at the IWG at ESTEC in January, 1996 and crew procedure development was initiated with the BGT. Draft GSRD and EPPs for SS/M-05 have been submitted to allow preparations to proceed for the EST scheduled for May. Ground support personnel have been hired and are now under training. Preliminary experiments on data analysis have been initiated. In conclusion, the progress of this year's tasks has been excellent and should provide NASA and ESA personnel with sufficient support to allow our planned experiments to be ready for EST, crew training, and flight.

In order for fertilization to occur, sperm must become motile and undergo a process termed capacitation prior to being able to fertilize the egg. Some male factors are correlated with a lower ability to undergo changes in sperm motility associated with capacitation. This area of sperm function has been studied for a long time under Earth gravity conditions and is of particular relevance to male infertility. The planning for long term space habitation raises the question of whether the normal fertilization produces might be altered under microgravity conditions. Earlier unmanned microgravity experiments, in fact, demonstrated that motility of bull sperm is altered significantly in microgravity. Our area of focus, in ground-based experiments, has been to study the role that second messenger pathways play in regulating the initiation and modulation of sperm motility. In this regard, changes in second messengers and protein phosphorylation are critical components of the regulation of sperm motility. Our microgravity experiments will expand these studies to determine whether the reported changes in sperm motility under microgravity are correlated with alterations in the intracellular messenger pathways and protein phosphorylation targets that regulate motility. Since sperm expend considerable levels of the biochemical energy supply to produce motility, these studies are also relevant to bioenergetics under microgravity conditions. Results of these experiments will not only expand our knowledge of the basic biological process of sperm function, but also address and area of biology relevant to extended habitation in microgravity.

Publications, Presentations, and Other Accomplishments:

Ahmad, K., G.E. Bracho, D.P. Wolf and J.S. Tash "Regulation of human sperm motility and hyperactivation component by Ca^{2+}, calmodulin, and phosphoprotein phosphatases." Arch. Androl., vol 35, 187-208 (1995).

Bracho, G.E. and J.S. Tash "Assaying protein phosphatases in sperm and flagella." Methods. Cell. Biol., vol 47, 447-458 (1995).

Biochemical Assessment of Stress in Cardiac Tissue

Principal Investigator:

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Congressional District: -

Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: BSP-017

Solicitation: 93 OLMSA-02

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Effect of Microgravity on Rat Masseter Muscle

Principal Investigator:

Jenkins

Phone:
Congressional District: -

Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: BSP-007

Solicitation: 93 OLMSA-02

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Effect of Space Flight on Adrenal Medullary Functions

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Funding:

Project Identification: BSP-012

Initial Funding Date: 1/95

FY 1995 Funding: $20,000

Solicitation: 93-OLMSA-02

Expiration: 12/95

Students Funded Under Research: 2

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

It is well known that microgravity conditions during space flight affect endocrine responses causing alterations in hormone levels, e.g., in the adrenal, pituitary, thyroid, and pineal glands. Thus, microgravity-induced changes in blood- and urinary-catecholamine (CA) levels have been observed, yet the cellular/molecular mechanisms leading to these changes are not known. We hypothesize that space flight might alter the expression and specific activities of the adrenal medullary CA synthesizing enzymes. Using a previous tissue sharing program we obtained evidence for a microgravity-induced decrease in the expression and specific activity of rat adrenal medullary tyrosine hydroxylase, the rate limiting enzyme of CA synthesis. We now propose to confirm and extend our findings on the effects of microgravity on the CA synthesizing system by using additional adrenal glands from animals flown in some previous and future Space Shuttle missions.

Specifically, we propose to 1) determine total CA contents, in particular assessing the ratios of epinephrine/norepinephrine in intact adrenal glands, using reversed phase HPLC with electrochemical detection; 2) measure the specific activities of three of the major CA synthesizing enzymes, tyrosine hydroxylase, dopamine-beta-hydroxylase, and phenylethanolamine-N-methyltransferase using radioenzymatic/colorimetric assays; 3) assess the amount of immunoreactive CA synthesizing enzymes by quantitative Western blotting; and 4) evaluate the differential gene expression of CA synthesizing enzymes by using semi-quantitative PCR and slot blot technology.

Our initial findings of a significant microgravity-induced decrease of tyrosine hydroxylase expression and specific activity are exciting by themselves from the basic science point of view. Moreover they might be consequential for space flight in general. However, before any definitive conclusions can be

drawn, our observations need to be verified and extended using a larger sample size and more elaborate controls. The present NASA Research Announcement provides the prospect for a timely, thorough follow-up to our initial studies.

In view of the extended delay of over two years, there has been no progress achieved so far on this project. The tissues themselves arrived in December 1995. However, with the tissues now in place, we intend to carry out the analyses as planned. We will investigate the effects of space flight on adrenal medullary function by analyzing tissue catecholamine contents by HPLC with electrochemical detection. We will analyze the expression/activity of catecholamine synthesizing enzymes and finally, we will check expression of the same enzymes by immunohistochemistry. Due to the long delay between writing the grant, obtaining the tissues and receiving all the funding in the near future, the team that originally was supposed to work on this project has dispersed. At this point the principal investigator is retraining two students in the lab to carry out the above analyses. The training will occupy much of the first three months of this year. In those three months, two students will be trained in analyzing catecholamines and assaying catecholamine synthesizing enzymes as well as in immunohistochemistry. Thereafter, we anticipate spending the remaining nine months on carrying out the experiments as described in the proposal.

Since this is a strictly physiological/medical study on animals flown in space there is no direct Earth benefit. On the other hand, our initial results published in FASEB J. suggested for the first time a decrease in catecholamine levels in rats that were directly exposed to space flight. More recently, published reports on human astronauts seem to confirm our experimental data. Therefore, the continued broadening of our studies will be of enormous importance for understanding in detail the mechanism of how and why catecholamine synthesis and secretion is decreased in vertebrates exposed to microgravity in space.

Mitochondrial Changes in Muscle Following Space Flight

Principal Investigator:

Martin

Phone:
Congressional District: -

Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: BSP-002

Solicitation: 93 OLMSA-02

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Glial Cell Reaction from Space Flight

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: BSP-008

Solicitation: 93 OLMSA-02

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Vitamin D Endocrine System After Short-term Space Flight

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Co-Investigators:

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Funding:

Project Identification: BSP-006
Solicitation: 93-OLMSA-02
Initial Funding Date: 9/94
Expiration: 12/95
FY 1995 Funding: $29,610
Students Funded Under Research: 1

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

We used immunohistochemical, biochemical and molecular approaches to analyze the expression of calbindin-D_{28k} and calbindin-D_{9k} in kidneys, intestine, and pancreas of rats flown for 10 days aboard the Spacelab 3 mission. We compared the effects of microgravity on calbindins in rats in space vs. "grounded" animals (synchronous Animal Enclosure Module controls and tail suspension controls). Exposure to microgravity resulted in a significant decrease in calbindin-D_{28k} content in kidneys and calbindin-D_{9k} in intestine of flight and suspended animals, as measured by enzyme-linked immunosorbent assay (ELISA). Immunohistochemical results demonstrated similar changes in calbindins in some animals. These findings suggest that a decreased expression of calbindins after short-term exposure to microgravity and modelled weightlessness may affect cellular Ca^{2+} homeostasis and contribute to Ca^{2+} and bone metabolism disorders induced by space flight.

Two species of rat calbindins, calbindin-D_{28k} and calbindin-D_{9k}, were measured using enzyme-linked immunosorbent assay (ELISA) with monoclonal anti-calbindin-D_{28k} antibodies and antiserum against calbindin-D_{9k}. Significant decreases in levels of renal calbindin-D_{28k} and intestinal calbindin-D_{9k} were found in flight and suspended animals. The level of renal calbindin-D_{9k} was very low and did not change significantly.

In situ levels of calbindins on fixed tissues were evaluated using described above primary antibodies and biotin-streptavidin techniques. Immunohistochemical results demonstrated are similar to those obtained with ELISA, changes in calbindins in some animals, especially in tail suspension groups.

In summary, the results obtained suggest that a decreased expression of calbindins after a short-term exposure to microgravity and modelled weightlessness, may affect cellular Ca^{2+} homeostasis and

contribute to Ca^{2+} and bone metabolism disorders induced by space flight. Decreased levels of intestinal and renal calbindins during a long-term space flight may significantly contribute to the development of disorders in Ca^{2+} and bone metabolism; however, whether this is the case remains to be answered.

The exposure of the body to microgravity during space flight causes a series of well-documented changes in Ca^{2+} metabolism, yet the cellular/molecular mechanisms leading to these changes are poorly understood. There is some evidence for microgravity-induced alterations in the vitamin D endocrine system, which is known to be primarily involved in the regulation of Ca^{2+} metabolism. Vitamin D-dependent Ca^{2+} binding proteins, or calbindins, are believed to have a significant role in maintaining cellular Ca^{2+} homeostasis.

The results of this study contribute to our understanding of the genesis of microgravity-induced disorders of calcium metabolism. Understanding of the mechanisms underlying those disorders are important to the development of pharmacologic and dietetic therapeutic strategies to prevent bone and calcium metabolic diseases in space and on Earth.

The Effect of Space Flight on Rodent Ocular Tissues

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Funding:

Project Identification: BSP-013

Solicitation: 93-OLMSA-02

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

The Effects of Space Flight on Bone Strength and Intracellular Calcium in the Rhesus Monkey by Non-invasive Techniques

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Funding:

Project Identification: 26-12-02 and 30-43-04

Initial Funding Date: 1992

FY 1995 Funding: $28,000

Solicitation: AO/Cosmos Program

Expiration: 1995

Students Funded Under Research: 4

Flight Information:

Responsible NASA Center: Ames Research Center

Task Description:

The purpose of this flight project was to apply noninvasive techniques for estimating bone function and calcium metabolism in individuals exposed to space flight through the Cosmos program. 1) The non-invasive technique that estimates bone function is an analysis of the response to a vibratory stimulus. This test uses a new instrument called the Mechanical Response Tissue Analyzer (MRTA) that originated at ARC through NASA basic research support. 2) The non-invasive technique that estimates intracellular calcium (ICCA) is an innovative test developed by a small company in the Bay Area. The test uses x-ray micro-analysis to determine the mineral and electrolyte content of sublingual cells that are fixed on special slides after they are obtained by gently scraping the surface of the oral mucosa. We had obtained sufficient preliminary data for both tests in ground based human experiments to justify their use in the non-human primates that were scheduled for the Cosmos 2229 mission.

The experimental design of the space flight project was to acquire measurements that used both tests, the MRTA and the ICCA at 3 or 2 time points in 2 monkeys pre-flight and post-flight. We treated the monkeys as single case reports whose pre- and post-flight measurements were evaluated against a background of normative data acquired in the Rhesus monkeys that grew up in the vivarium in Moscow. We had also acquired longitudinal data in a pilot study for this space flight beginning 2 years prior to the flight in order to determine whether chair restraint per se caused changes in the test results.

An additional consideration in this project was the need to evaluate the nutritional status of vitamin D in Rhesus monkeys at ARC and in Moscow. Vitamin D metabolism in this species is different from the human with respect to the circulating levels of the vitamin D hormone, 1,25-dihydroxyvitamin D. We measured the hormone and its substrate in the blood of ARC animals fed commercial diets heavily supplemented with vitamin D and in Russian IMBP monkeys fed natural food without supplements.

The non-invasive test of cross-sectional bending stiffness with the MRTA has proven to be a valid biological direct measure of the biomechanical properties of the tibia. Current practice is to determine the functional integrity of bone by quantifying the mineral content, an essential component to the strength of the bone. The non-invasive MRTA test result may be a more complete measure of the functional integrity of a long bone and avoids exposure to radiation. Additionally, we found reduced bending stiffness in the tibias of monkeys who were restrained in chairs only 2 weeks. To document significant changes in a biomechanical property of bone after so short a time interval was unexpected and emphasizes the dynamic nature of bone. The space flight results differed from chair restraint on the ground in that post flight values in 2 animals who lost weight did not change more than 2 weeks after landing. Our findings suggest that depressed bending stiffness so soon after chairing on the ground is more likely related to metabolic than structural elements affecting mechanical properties in bone.

Comparison of sublingual cell concentrations in 2 groups of non-human primates with different diets revealed little connection between the intracellular ion level and the level of ion in the diet except for potassium whose concentration more or less paralleled the dietary intake of the ion. In chaired monkeys, there were increases in the levels of the ions normally in the bone, Ca, Pi and K, an indication of the effect of chair-restraint on the transport of ions. Ion levels after space flight were similar to those in chair restrained animals suggesting that restraint and inactivity, rather than microgravity accounted for the changes in ion levels.

The Mechanical Response Tissue Analyzer (MRTA) represents advanced technology that has application not only to loss of bone during space flight but also to any situation or condition in which the functional integrity of bone (i.e., its strength and mechanical properties) is in doubt. Currently, physicians use bone scans by densitometers to determine bone mass recognizing that the lower the mineral content, the more fragile the bone. In recent years, the failure of bone mass measures to predict fractures has given rise to an interest in techniques that would permit some evaluation of bone structure as well as mineral. The MRTA, quantifies bending stiffness or the intrinsic rigidity of the bone, a function of both its mineral content and its structure (geometry). This information can be of value especially in the practice of medicine to the Orthopedist, the Rheumatologist and most importantly to the internist to detect and monitor patients with osteoporosis as a consequence of age or menopause or both. The MRTA has the advantage of detecting the response of bone at its natural frequency with an instrument that is of low cost, small size and easy transportability. The instrument is currently available for research studies involving the human ulna, but analysis of the human tibia remains experimental.

Publications, Presentations, and Other Accomplishments:

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Effects of Microgravity on Quail Eye Development

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Funding:

Project Identification:

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FY 1995 Funding: $60,219

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Task Description:

During embryonic development, the most exposed tissue of the eye, the cornea, becomes differentially bulged outward because of constant intraocular pressure (IOP). The component cells of the cornea secrete a unique, paracrystalline extracellular matrix (the stroma) composed of orthogonal plies of collagen fibrils and proteoglycans. The cornea remains avascular, becomes transparent, and becomes more densely innervated than any other region on the surface of the body. Corneas from chicken embryos that flew on STS-47 contain many more cellular processes in the outermost region of the stroma (Bowman's Layer) than any corresponding region of control corneas. These processes appear to be cross-sections of cytoplasmic extensions of cells and are found in that region of Bowman's Layer immediately beneath the basal lamina of the corneal epithelium. Here, we propose to compare corneas of quail that flew in space on Mir-1 with those of ground controls to determine if the same unusual cellular processes are seen as in the space-flown chicken corneas. In the central regions of such space-flown corneas, the processes appear to be either portions of basal epithelial cells whose pseudopodial extensions have migrated down through their own basal lamina into the stroma, or corneal nerves that have innervated the corneal stroma in an unusual manner. Eyeballs of embryos fixed on Mir-1, control embryos fixed at KSC and clinostated embryos fixed at KSU, will provide corneas for this study. Electron microscopy will be used to assess the distribution of the cellular processes in Bowman's Layer in the central region of each cornea. Attempts also will be made to determine the relative glycosaminoglycan distributions in the corneal stromas by indirect immunofluorescence and to record whole-mount staining patterns of the corneal nerves.

On two separate flights to the Mir space station on which quail eggs were incubated in a Flight incubator (Mir-18/STS-71 and Mir-19/STS-74), virtually all of the embryos died after 4-5 days of incubation. Because the same pattern of early mortality was observed when quail embryos from the

same batch of eggs were incubated in an identical flight-model incubator on Earth, and yet because embryos from the same batch of eggs developed normally when incubated in a standard laboratory-model incubator, it appears that the design of the flight-model incubator must be changed before the flight experiment can really be performed.

Despite this disappointment with the equipment, some information has been obtained. Quail embryos, particularly from the breed developed by the Russian space agency, appear to be quite hardy, able to withstand the stresses of transport to the launch pad, the launch itself, and incubation on Mir. In addition, it appears that the cosmonauts did an excellent job of transferring the quail eggs from the incubator to the ziplock bags of fixative solution and breaking the shells gently to allow fixative penetration, all without release of fixative solution into the cabin. Return of the fixed eggs from Mir to the U.S.-Shuttle, and from the U.S. landing site to NASA-Ames Research Center, all occurred as planned, as did matching fixations performed on Earth on the two control groups of embryos - those incubated in the Flight-model incubator and in the laboratory-model incubator.

In our own work, we have determined that the corneas of embryos stored for at least 4 wks at room temperature in the same type of 4% paraformaldehyde solution as used on the Flight and control embryos, when followed by further fixation in glutaraldehyde and osmium tetroxide, show excellent preservation of ultrastructure in the extracellular matrix and intracellularly. Thus, we are confident that we will be able to compare the ultrastructure of corneas from flight, synchronous ground controls (flight-model incubator), and ground (laboratory-model incubator) controls.

In addition to recording eyeball weight, volume, and diameter and cornea diameter, transparency, and ultrastructure, we appear to be close to having an operational method that will allow us to perform whole-mount immunostaining of the nerve patterns of corneas, even after long-term storage at room temperature in paraformaldehyde.

Several questions have been answered, such as: the flight-model incubator hardware is not designed optimally, quail embryos are excellent models for this experiment, Russian and U.S. flight and ground personnel can perform all operations as planned, both in flight and on the ground, fixation protocol produces eye tissues fixed acceptably for our analyses, an alternative fixative solution (85% ethanol/15% glycerol) was tested on flight and control embryos and shown to give tissue fixation that was very poor, in comparison with fixation by 4% paraformaldehyde.

Could an already-Flight-tested type of avian egg incubator be substituted for the current Flight-model incubator on the Mir station? For example, such an incubator is available from SHOT, Inc. (Space Hardware Optimization Technology, Inc./P.O. Box 351, Floyd Knobs, IN 47119/Vice- President: John C. Vellinger/Tel: (812)-923-9591/FAX (812)-923-9598). This model incubator was used successfully for incubating chicken eggs on STS-29, and chicken eggs are much larger, and encased in more brittle shells than those of quail. Retrofitting this incubator for incubating quail eggs would not be difficult mechanically. Whether it could fit into the space currently occupied by the quail egg incubator on Mir, and operate on the electrical power source available there, would have to be discussed by Russian and U.S. engineers.

A method is needed for providing a continuous, reliable recording and print-out of incubator temperature and relative humidity during the entire incubation period in the flight incubator, and in both types of control incubators on Earth as well. A comparable system also should be devised for monitoring temperature and relative humidity during prelaunch storage and transport, as well as during egg storage during launch and transport to Mir.

We would like to devise a technique for monitoring the intraocular pressure of embryonic quail eyes during incubation in microgravity, in comparison with incubation on Earth.

We will not be able to perform this experiment unless a Flight-model incubator, suitable for use on Mir, can be designed quickly and be demonstrated to provide as optimal an environment for incubating quail eggs as provided by currently available laboratory-model incubators. Such comparisons should be done first on Earth, repeatedly, to demonstrate the long-term reliability of the new Flight-model hardware. For comparison, it is worth noting that many developmental biologists have simple, laboratory-model avian egg incubators that have been running continuously in their labs for periods exceeding 25 yrs, serviced only by a monthly drop of oil! Such incubators can be used for incubating chick and quail eggs simultaneously.

Until new Flight-model incubator hardware is assembled and tested successfully on Earth, justifying further attempted incubations on Mir, we will use the time and resources available to try to increase the amount of information that we will be able to obtain from eyes of quail embryos stored in paraformaldehyde fixative solutions at room temperature. For example, these efforts have already provided us with an exciting new method for visualizing the whole-mount nerve patterns of entire quail corneas. Corneas are the most highly innervated structures on the surfaces of vertebrate bodies, so being able to visualize their nerve patterns easily in heavily- fixed tissue is a major accomplishment.

The eye is dynamic in at least two respects. First, the eyeball itself develops during embryogenesis much like a balloon or and 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.

Skeletal Development in Long Duration Spaceflight

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 6/95

Expiration: 5/97

FY 1995 Funding: $64,816

Students Funded Under Research: 1

Flight Information:

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.

Project funding began in May 1995 and the first samples from Mir were received and dissected at Ames Research Center (ARC) in July 1995. We have had a subsequent dissection from a second flight, which also occurred at ARC in December 1995. Four different development periods were studied at embryonic ages 7, 10, 14 and 16. Unfortunately in both Mir flights there was a malfunction of the egg incubator resulting in limited viability of these embryos. We collected samples from the flight, synchronous controls and lab controls. We are in the process of studying these samples using light and electron microscopy, histochemistry and immunocytochemistry as outlined in our original proposal. Because of the relatively short period of time between the collections and the present report, we do not have any conclusions about the study at this time.

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.

Effect of Microgravity on Afferent Innervation

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 9/95

Expiration: 9/96

FY 1995 Funding: $94,497

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Task Description:

This proposal will 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 the quail (*Coturnix coturnix japonica*) raised in space. One ear of birds available through this NRA will be used to: I. 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-100ß 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-100ß 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 μm. 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.

Specimens available for morphological analysis to date were not sufficient for comparisons.

It appears that specimens exposed to microgravity may suffer alteration in the geometry of the posterior and horizontal semicircular canal topography. This observation, if validated by examination of synchronous and normal ground controls, would be significant because endolymphatic flow will be altered with a net effect on the stimulation of the hair cells in the cristae of these canals. Electron microscopy analysis of specimens is still in progress. Immunohistochemical analysis of specimens is still in progress.

The research proposed in this application does not seek to develop new therapeutic treatments for use at the 1.0G earth environment. The research will,however, provide invaluable information for better understanding the functioning of the vestibular (balancing & 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 1.0G certain conditions of the space environment can not be replicated for long time. Only long time 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 1.0G are known to depend stimulation aided by the force of the gravity vector. There is 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 1.0G environment of the Earth.

a) Can vertebrates developed in space without otoconia and function normally at 1.0G 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.

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Effects of Weigtlessness on Vestibular Development

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Funding:

Project Identification:

Solicitation: NRA 93 OLMSA-05

Initial Funding Date: 7/95

Expiration: 6/96

FY 1995 Funding: $82,736

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Task Description:

This experiment addresses the question of whether gravity is essential for the normal development of connections between the gravistatic ear and the brain in quail. Previous work suggests that chicken raised in microgravity have difficulties orienting in space and this up to a point where they may not be viable. We suspect that this is due to abnormal formation of connections between the developing ear and the developing brain. This experiment will test the outcome of a long-term exposure to microgravity on the development of inner ear connections in quail embryos. The data are valuable extensions of previous work on developing rat fetuses for which it is difficult to obtain such a long term exposure.

An integral part of our project is the successful incubation of embryos from a stage before the inner ear fibers are growing into the brain (e.g., approximately 3 days of incubation) to a time prior to hatching (about 14 and 16 days of incubation), a task very difficult for the alternate model, the rats. Thus far, two missions were flown successfully for the appropriate time. However, none of these missions has provided material for us owing to mishaps during incubation of the quail eggs. It appears now that the redesigning of the incubator has been completed for the eggs and we are looking forward to obtaining microgravity-exposed quails.

Thus far, we have fine-tuned our application of the tracers to the ear of control animals. In the process of doing this we have obtained valuable insights into a long-neglected issue of inner ear connections, collateral fibers interconnecting between different sensory epithelia. We hope to complete this set of information later this year and publish it. If we can obtain equally well-preserved flight quails, we can certainly obtain the requested information for our project. We are very much looking forward to our next landing of samples, our major task for the near future.

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.

Publications, Presentations, and Other Accomplishments:

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Hypogravity's Effect on the Life Cycle of Japanese Quail

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Funding:

Project Identification:

Solicitation: NRA

Initial Funding Date: 6/95

Expiration: 5/96

FY 1995 Funding: $55,007

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Task Description:

Quail eggs of the hypodynamic strain were imported from Moscow and are currently incubating in quarantine. Hatchlings will remain in quarantine for 30 days post hatch and at that time released by USDA if proven to be disease free. The hatchlings will be grown to adulthood. Fertile eggs of the hypodynamic strain will be express mailed to Kennedy Space Center to be flown on STS-76. The shuttle will link with Mir where eggs will be transferred to the Russian egg incubator for development.

It was originally intended to test this hypothesis using egg incubation hardware similar to that used on Mir. However, due to the lack of availability of Russian hardware for ground based studies thus far, experiments were conducted using a laboratory incubator. Quail eggs turned 0, 1, 2, 3, and 4 times daily showed hatchability percentages of 63, 66, 69, 75, and 76%, respectively. Results of this first experiment indicate that quail eggs should be turned at least three times daily to improve the rate of hatch. Chickens eggs were also included in the study and their hatchabilities were 26, 49, 56, 58, and 64% for 0, 1, 2, 3, and 4 rotations per day. These results indicate that chick embryos are more adversely affected by lack of turning than quail eggs and that they need to be turned at least 4 times daily or more for improved rate of hatch.

Results indicate that microgravity may be needed for the earliest stages of embryogenesis. However, more reliable egg incubation hardware is needed with a centrifuge before this hypothesis can be adequately tested. Preliminary results indicate no adverse effects of vibration and g force (launch profile of the shuttle) on avian development. Only five quail embryos have survived microgravity and only one of these embryos survived to the latter stages of development (15 days of incubation). Therefore, interpretation of results on utilization of minerals from the shell will be difficult. Analysis of shells is currently in progress.

Research will help to elucidate the role of microgravity in the development of the avian embryo. Results of flight experiments indicate that gravity may be needed during the earliest stages of avian embryogenesis, but is not important for the latter stages of development. An egg incubator with centrifuge on the International Space Station or the shuttle will determine if lack of gravity is the reason for the death of young avian embryos in space.

Effect of Microgravity on Osteoblast Gene Expression

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Funding:

Project Identification:

Solicitation: NRA 94 OLMSA-03

Initial Funding Date: 1995

Expiration: 1999

FY 1995 Funding: $193,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Euro-Mir

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 impediments 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 proposal aims to analyze how microgravity effects 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 E_2 (PGE_2) from osteoblasts. PGE_2 can increase trabecular bone formation in rats. The lack of PGE_2 synthesis occurring in space may be a critical factor responsible for the bone

loss that occurs in astronauts. PGE_2 down regulation 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 PGE_2 synthesis and action; c-PLA_2 (cytosolic phospholipase A_2), COX-1 and COX-2 (cyclo-oxygenases), and the PGE_2 receptors EP_1, EP_2, and EP_3. 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.

Preliminary testing and an activity plan for Biorack for first year (Jan 1995-December 1995) have been completed.

1. Biocompatibility testing in modules Test for viability under conditions for all material types to find best combination

A. Media: Keep media interface for specified times and then add to growing cells to test for toxicity by measuring viability of cells at RT for 12, 24 and 36 hours and compatibility with various materials for 12, 24, 36,48, 3, 4, 6, and 8 days by the methods below:
RESULTS: No Toxicity

B. Cells: Keep cells in modules for specified times and test for toxicity by measuring viability of cells at RT for 12, 24 and 36 hours.
RESULTS: No Toxicity

C. Fixative I: Leave fixative on modules with cells for specified times, freeze and test for recovery of RNA in samples using extraction and verification of purity by agarose gel.

1)Recovery of samples after frozen for 1, 2, 4, 6, and 8 days.

2)Compatibility of fixative with module materials for 12, 24, 36, 48, 3, 4, 6, and 8 days (will compound damage unit material and/or is it still effective).
RESULTS: RNA recovered from cells grown in plunger modules.

D. Fixative II: Leave fixative on modules with cells for specified times, freeze and test for fixation in samples by visualization of actin and surface receptors

1) Recovery of samples for 1, 2, 4, 6, and 8 days.

2)Compatibility of fixative with module materials for 12, 24, 36, 48, 3, 4, 6, and 8 days.
RESULTS: Lexan material of plunger is slightly discolored, but RNA OK.

2. Loading and Launch Delays Experiment Start-time Delays

A. Test for recovery of cell growth in 0.5% sera after delays of 12, 24, 36, 48, 72 and 96 hours. test for viability by measuring viability of cells Using

1) cell count and

2)bromophenol blue exclusion and glucose utilization using aMEM, and aMEM with Hepes
RESULTS: Cells are activated and start new growth at appropriate time period.

B. Test for recovery of gene expression in cells activated with 10% FCS in sera after being in 0.5% sera after delays of 12, 24, 36, 48, 72 and 96 hours using rtPCR. Genes tested, c-fos, COX-2, actin and cyclophilin.
RESULTS: Cells are activated and start new growth at appropriate time period, RNA is being analyzed.

3. Crew procedures must be developed, verified and written COMPLETED.

4. Launch profile G forces effects on gene expression:

A. Using facilities at AMES, we will take the cells in the modules through launch profile with 'ground control' and then test for changes in gene expression. This data will tell us if the forces of launch will cause any activation of gene expression, if so, activation will be delayed in orbit.
RESULTS: Initial studies revealed problems in activation spring, problem was resolved and testing is planned in early 1996.

5. EST simulation at KSC, get first ground base results on
1) cell growth
2) mRNA expression during experimental protocol on c-fos, cyclophilin, COX 1 and 2, $cPLA_2$, statin, PCNA and EP_1, EP_2, and EP_3 receptors.
3) photography of cells fixed in formaldehyde using actin, Hoescht dye.

Osteoporosis is a generic term used to describe various bone diseases that are manifested by resulting in fractures of the vertebrae, wrist, hip, humerus, and tibia. Osteoporosis is common in older adults, in the presence of glucocorticoid excess as in Cushings syndrome and in people treated for asthma with steroids. Osteoporosis has also been noted in healthy astronauts that are in microgravity for extended duration. Our studies are concentrated on the basic mechanisms that regulate new bone growth and the relationship of growth to drugs and environment. In our flight studies we will find the basic signals which will increase bone growth and formation and compare the gene expression and cell morphology in microgravity and 1-g environment on Biorack .

Asthma patients, Cushing patents, and astronauts that have osteoporosis have one thing in common, an increase in glucocorticoids. After analysis of Skylab data, we have 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 PGE_2. We have also found in our flight experiment on STS-56 that microgravity interferes with normal bone cell growth activation and causes reduced PGE_2 synthesis, that observation is in press in *Experimental Cell Research*. In addition, 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 PGE_2. 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 PGE_2 from the bone cells which is followed by elevated gene expression of cyclo-oxygenase which is needed for bone growth. This is probably the major mechanism by which exercise augments bone growth (manuscript in preparation).

The new technology made possible by our NASA grant have 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.

Publications, Presentations, and Other Accomplishments:

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Avian Blood Formation in Space

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Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $66,720

Students Funded Under Research: 1

Flight Information:

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.

According to the award letter the grant is active as of Sept 1, 1995. Nevertheless, we have carried out in 1995, at our own expenses, significant studies both with ground-based controls as well as with samples returned from MIR 18. At this point in time we've evaluated only some of the ground based controls. The following progress has been achieved so far: 1) We have optimized the fixation techniques for quail embryos by introducing multiple hairline fractures in the egg shell which permit rapid penetration of the fixative. These techniques can also be implemented during flight and the crew for MIR 19 was instructed in these techniques. 2) We have successfully immunostained blood vessels (endothelial cells) in chorioallantoic membranes (CAMs) isolated from quail eggs after 2-4 months fixation, thus providing evidence that long-term fixation under conditions encountered aboard the MIR space station will not necessarily abolish antigenicity. However, we found that, depending on the antigen studied, immunoreactivity is partially impaired, thus requiring laborious optimization of the staining procedure for each individual antigen. 3) By using eggs (courtesy of Dr. P. Hester, Purdue University) from the same hypodynamic strain that is used in the flight experiments, we have established important baseline values for evaluating angiogenesis during the development of the vasculature in the quail CAM. In addition to the immunological procedures detailed in the proposal we developed novel fluorescent techniques to visualize blood vessels in whole mounts of long-term fixed tissues. The latter procedure allows us to carry out complex, computer-aided morphometric analyses. To the best of our knowledge, such elaborate studies have not been carried out before in quail CAMs. The results from this study, currently being compiled for publication in a peer-reviewed journal, will serve as ubiquitous comparators for assessing the effects of space flight on vascular development. We validated the usefulness of our "gold-standard" data by comparing them to the normal laboratory controls for the MIR 18 experiments: The vascular development, as assessed in terms of blood vessel density, branching, etc., was identical for eggs incubated both here and in Moscow.

We participated in the dissection of the eggs returned from MIR 18 and, most recently, also from MIR 19. To study the effects of space flight on embryonic/fetal development, the investigators' group decided to have the eggs fixed at days 7, 10, 14, and 16. Disappointingly, none of the flight eggs showed significant embryonic development. Moreover, many of the synchronous controls, in particular, those of the MIR 19 experiments, were "underdeveloped" or had died prematurely. Preliminary data can be summarized as follows: a) In both experiments, the state of tissue preservation

(fixation) was not always adequate. The problem of appropriate fixation needs further attention. b) To date we have evaluated in part the returned CAMs and adrenals from the laboratory and synchronous controls of MIR 18. As indicated above, the laboratory controls developed normally. By contrast, we detect developmental anomalies in the vasculature of all usable CAMs, i.e. those that seemingly developed to term (days 7-16). Surprisingly, even in those CAMs, we see a lack of normal vascular development. These preliminary data suggest that during the launch and/or subsequent incubation, the eggs must have experienced certain, as yet, unknown conditions, which thwarted normal development. One preliminary conclusion is that in early embryonic development the establishment of the extraembryonic vasculature is prone to damage by adverse environmental constraints such as launch/incubation conditions. The investigators' group is currently discussing how to remedy some of the potential problems in the upcoming flights.

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.

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Funding:

Project Identification:

Solicitation:

Initial Funding Date:

Expiration:

FY 1995 Funding: $140,000

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir-94

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

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Funding:

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Solicitation:

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir-95

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Effects of Weightlessness on the Avian Visuo-Vestibular System: Immunohistochemical Analysis

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 6/95

Expiration: 5/97

FY 1995 Funding: $62,031

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Task Description:

The purpose of the proposed 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 will be analyzed by using immunohistochemical techniques. Brain tissues from quail which are developed in space will be used.

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 will be studied in quail.

Because of the time and spatial limitations as well as the safety precautions in Mir, the procedures for tissue fixation will not be ideal for histological studies. However, preliminary studies indicated that

the fixation under such conditions produces reasonable staining results. Furthermore, in order to share the limited number of specimens with the other principal investigators of Russia and the U.S., after the tissues are brought back to Earth, the brains will be dissected into small pieces which will be processed separately. This project will thus concentrate on the analysis of the forebrain whereas the lower part of the brain will be analyzed by other principal investigators.

Two flight experiments were completed in 1995 (Mir 18 and 19). Because of technical problems with the incubator in Mir, only a few quail embryos have been recovered from the flight experiments. Nevertheless, data from control groups and a limited number of data from the experimental groups have indicated that an immunohistochemical analysis can be successfully done using the brain tissues that were fixed in space.

Twenty-seven tissue samples have been received from the lab control groups, 6 from the synchronous controls, and 4 from the flight groups. Among them, some tissues were not well fixed at all and thus could not be processed for a histochemical analysis. Tissues which were well fixed have been analyzed histochemically. We have completed the analysis of 8 tissue samples, including 1 tissue sample from the synchronous control group and 1 tissue sample from the flight group. The results of the analysis indicate that these tissues can be stained with many neurochemicals despite the not-ideal fixation procedures in space. In particular, calcium-binding proteins (i.e., parvalbumin, calbindin, calretinin) were most clearly detected in all the tissues. Since these chemicals are important neuroanatomical markers, they will be useful for detecting morphological and chemical changes in the development of the neural system in space. The staining processes using antibodies against several calcium-binding proteins worked very well in the tissue samples. These chemicals are closely associated with the development of the avian neural system. Are other antibodies which are associated with neural development also good markers? Tests will be conducted using additional antibodies (e.g., calmodulin and neurophysician) to further the goals of the study. The results have thus far supported the use of the tissue fixation procedures and have identified some neurochemicals which are ideal for an immunohistochemical analysis under such procedures.

Visual deprivation may cause defective growth or development of neurons in the visual pathways and the cortex in terms of cell morphology, connections, and chemistry. Little is known, however, about how vestibular deprivation affects the fundamental nature of the development of the neural mechanism. Without the experience of normal gravity, are the neural structures of the visuo-vestibular interactions able to develop normally? Are particular cell groups more susceptible to the deprivation of gravity? An examination of the distribution of a variety of neurochemicals during development (of both in-space and on-Earth samples) will give an important answer to these questions.

Fecundity of Quail in Spacelab Microgravity

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Co-Investigators:

Funding:

Project Identification: NAS2-14213

Solicitation: 93-OLMSA-06

Initial Funding Date: 5/95

Expiration: 4/96

FY 1995 Funding: $57,460

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: Ames Research Center

Flight Hardware Required: Incubator II Joint US/Russian

Task Description:

We hypothesized that a regenerative life support system can be provided in the "Spacelab Mir-1" for the Japanese quail. We proposed to test this hypothesis with three objectives designed to determine the effect of microgravity on 1) embryonic development initiated after the launch; 2) the fecundity of adult quail during orbit and the assessment of their hormones and reproductive tissues after orbit; and 3) the regeneration potential of quail in microgravity based on primordial germ cell migration and differentiation, gametogenesis, ovulation, fertilization, embryonic development, and hatching.

The experiment proposed will provide substantial basic information about the effects of microgravity on embryonic differentiation and development, as well as important information about adult quail endocrinology and physiology. Many aspects of the proposed work will focus on reproduction since this is the only path for successful animal bioregeneration in space.

A substantial amount of work was accomplished in 1995 toward Objective #1. The Mir-19 and 19 flights demonstrated adequate procedure for the delivery of fertile egg to the Mir Space Lab. Crew tasking for incubator monitoring, embryo fixation and return of the fixed eggs by either Soyuz or Shuttle were shown to be practical.

These two flights have clearly shown that on ground and preflight tasks were performed in a scientifically feasible manner. The egg container used in the launch and return was adequate to accomplish the planned mission. Also, a key component was establishing fixation bags containing paraformaldehyde as an acceptable procedure by the PIs. The breaking of the shells during placement in the fixative by the Mir crew was a critical step to allow proper embryo fixation. As one of the two PIs

that travelled to the Moscow lab, Dr. Wentworth developed a greater appreciation for the requirement of servicing the existing Mir-Lab incubation equipment and also the synchronous equipment at the ground lab in either Russia or the United States.

We conducted critical ground experiments and developed an easily formulated 70% water gelatin based quail diet (NASA-GEL) that contained all the required nutrients. These data demonstrated that the birds on the NASA-GEL were able to maintain adequate hydration, and their body weight, egg production, fertility and hatchability of fertile eggs equaled or surpassed that of the controls. This may prove to be a more manageable system than the currently proposed liquid diet for adult quail. Also, ground experiments are in progress to evaluate egg storage at four and 13°C in a dry and PBS environment. Egg storage studies have not been completed at this time. Additionally ground experiments are being conducted to determine at 1 g the effect of no turning verses turning eggs 180° every four hours on embryogenesis through to hatching.

The results of both flights consisted of early embryonic death with only two embryos living to the 16th day of incubation. The interpretations of results were made more difficult by the fact the synchronous control showed a similar lack of viability. Retrospective analysis of onboard flight recording data suggests that the incubator temperature control malfunctioned and the eggs were being incubated at 42°C instead of the programmed 37.5°C. A question that must be resolved is the inclusion of accurate in-flight temperature and humidity recorders. A recent ground control run at the Ames Lab suggests the problems in flight are not associated with the vibration and g force of launch.

Preliminary histological evaluation of the embryonic reproductive organs of the viable inflight embryos suggest normal development. Critical stages of development from heat stressed embryos appear to be at days 1, 3, and 5.

We believe that these 1995 preliminary studies pave the way for an expected highly successful flight in March 1996 on STS-79. We look forward to the flight of adult quail, and we expect that they will lay fertile eggs as a result of the crew performing artificial insemination.

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 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.

In trials to be done with adult quail, we will determine the normality of endocrine and physiological processes as related to avian reproduction function in microgravity. We anticipate that with controlled light (14 hr/ 24 hr) reproduction will be normal exceptating process. The space environment will require that birds be artificially inseminated to obtain fertile eggs. There will be a pre-flight and post-flight comparison of endocrine profiles of the adult quail programmed on a flight scheduled in 1996.

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 1) the gelatin ration (which contains both solid nutrients and total water) as well as 2) extended holding periods for fertile eggs in a liquid environment to prevent dehydration before incubation.

Pulmonary Function During Extended Exposure to Weightlessness (Euromir 95)

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Funding:

Project Identification: Euromir '95

Solicitation:

Initial Funding Date: 7/94

Expiration: 6/96

FY 1995 Funding: $31,000

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir-95

Responsible NASA Center: Johnson Space Center

Task Description:

The lung is extremely sensitive to gravity, and experiments we performed on Spacelabs SLS-1, SLS-2 and D-2 showed marked changes in pulmonary function in microgravity. Using the RMS-II, we will study the distribution of ventilation and changes in rib cage and abdominal motion on one astronaut and one cosmonaut over a 4-6 month period in μg.

As an adjunct to this study, we will study the effect of raised CO_2 levels on pulmonary function in 4 subjects exposed to 23 days of raised environmental CO_2 levels of 1.2% and 0.7%. This study will occur in a chamber at DLR in Cologne, Germany. Note that this program is supported by travel money only.

1) Euromir 95:

All baseline data preflight have been collected, and inflight data collection is essentially complete (scheduled landing 2/29/96). Post flight collection is yet to occur. Equipment on orbit has functioned well except for some problems during the later portions of the flight. Full analysis is not yet possible as only limited data downlink is possible from Mir. Thus analysis awaits return of data from orbit.

2) CO_2 Exposure:

Both exposure levels have been completed. Few changes were seen at the 0.7% level, but some significant changes occurred at 1.2%. Data analysis is ongoing and a publication will be forwarded to Aviation Space and Environmental Medicine in the next few months. The results are summarized in the American Thoracic Society abstract included in this report.

The effect of long-term exposure to μg on the lung is completely unknown. Degradation of the respiratory muscles may occur and the exposure to inhaled particles increased. This provides a unique opportunity to study these effects on the healthy lung. The results will further the basic understanding of human pulmonary physiology.

The long-term exposure to low levels of CO_2 seen in space flight and in this ground-based study should shed light on the fundamental control of ventilation mechanisms present in man. Such knowledge is essential in environments such as Space Station where maintenance of very low CO_2 levels could become prohibitively expensive.

Microbial Cellulose Assembly in Microgravity

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Funding:

Project Identification:

Solicitation: 93 OLMSA-05

Initial Funding Date: 8/94

Expiration: 7/95

FY 1995 Funding: $99,835

Students Funded Under Research: 0

Flight Information:

Flight Assignment: BRIC-05 (STS-70, 6/95)

Responsible NASA Center: Kennedy Space Center

Task Description:

An apparatus has been designed and tested which is suitable for transporting cells of *Acetobacter* into near-Earth orbit aboard the Shuttle. The apparatus is passive and requires no intervention on the part of the astronauts. Three independent seals assure the containment of the experiments involving liquid media. No hazardous chemical or materials are employed and *Acetobacter* is non-pathogenic. If it is necessary to put the experiment on hold prior to launch, it is possible to put both bacteria strains in stasis in the cold for a maximum of four days without jeopardizing the experiment. In addition, the experiments can be rapidly re-initialized if necessary. By employing two strains, each cultivated in liquid and on solidified medium, the opportunity to observe effects of microgravity on cellulose synthesis is increased. In addition, cultivation liquid will permit synthesis of sufficient cellulose in orbit for a variety of post-flight physical and chemical analyses, including transmission electron microscopy, light microscopy, NMR, and degree of polymerization.

Previous research has indicated that the structure of the cellulose ribbon biosynthesized by *Acetobacter* during a flight of the NASA Reduced Gravity Laboratory was altered. However, due to the limitations of reduced gravity research conducted at or near the surface of the Earth it was not possible to unequivocally correlate these alterations with hypogravity. We have initiated ground-based research exploring different cultural approaches of *Acetobacter* that might be used in the Biological Research in a Canister (BRIC) system developed for space flight testing in the Shuttle middeck.

Two strains of *Acetobacter* were selected for ground-based work, AY-201 and NQ-5. Studies utilized two BRIC units (120 mm diam. and 312 mm long) capable of holding nine standard size polycarbonate Petri dishes. Petri dishes were sealed with two layers of plastic film and placed into the BRIC and incubated at 28°C. For cultivation it was necessary to devise a means of containing the liquid medium that would minimize agitation of the cells and hence maximize the opportunity to detect subtle

microgravity effects on cellulose morphology. The culture system also had to be autoclavable, lightweight, and permeable to oxygen.

Several designs were considered and tested, with the most satisfactory configuration being a disk-shaped silicone bag secured within a polycarbonate Petri dish. Two disks (0.02 mm thick medical grade silicone sheeting) were sealed together at their circumferences to form the bag and then suspended in a polycarbonate Petri dish by means of two acrylic rings. The bags could then be injected with a cell/medium mixture and mounted in a Petri dish and opened by cutting to remove the product with minimal disturbance. *Acetobacter* has been successfully cultured for 7 days in this apparatus in the BRIC system. Additional tests showed that the bacteria could be held in stasis in the system with cold temperatures, providing a means of dealing with launch delays.

Gravity and Bone Growth

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Funding:

Project Identification:

Solicitation: 93-OLMSA-07

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $174,075

Students Funded Under Research: 6

Flight Information:

Flight Assignment: Ground Definition

Responsible NASA Center: Ames Research Center

Flight Hardware Required: AEM

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 first grant year, two hindlimb suspension studies were completed. Although a third study was planned, animal research at Ames was halted and prevented that study. During the period when animal research was halted, we analyzed some remaining bones (femurs) from the SLS2 and PARE.3 experiments. Our initial conclusion from existing suspension and flight data that mRNA levels for TGFß and bone matrix proteins from cancellous bone are not significantly influenced by reloading following unloading was shown to be incorrect not only because of the initial standardization problems, but also because cancellous bone responds more rapidly than cortical bone periosteum to reloading. Following reloading after suspension, a suppression of some values is noted in the periosteum while the cancellous bone shows increases that peak at 8hr in type I collagen (ColI), at 12hr in transforming growth factor beta (TGFß), or at 16hr in osteocalcin (OC) after reloading. The effect on TGFß and OC in cancellous bone appears to be over within 24hr while the ColI effect is not. However, if the data are normalized to the maximum response, then the TGFß response is the first to

increase, followed by ColI and OC, suggesting that the growth factor is the first signal in this sequence. Body mass increased during the 9d experiments; no significant differences were noted between suspended rats and their corresponding control group. If anything, suspension appeared to slightly lower the ionic calcium without influencing pH. Over the 9d experiment, control animals showed about a 15-20% decrease in bone mineralization rate while the suspended animals slowed their periosteal growth rate by about 40%. No significant difference in medullary area or femoral length was noted among the groups at any time point. In conclusion, the cancellous response is more rapid than the cortical response to reloading. Elevation of steady state message levels in cancellous begins as early as four hours following reloading, while the periosteal response is not apparent until about 24hr. As with the cancellous bone, TGFß appears to increase first followed by OC and ColI at the periosteal surface.

During the first grant year, two hindlimb suspension studies were completed. Although a third study was planned, animal research at Ames was halted from 3/23/95 until 6/7/95 and prevented that study. During the period when animal research was halted, we analyzed some remaining bones (femurs) from the SLS2 and PARE.3 experiments. The studies 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 dose respond to reloading and, in fact, responds more rapidly than cortical bone (see abstract). We found suggestions that the initial stimulus may be TGFß followed by elevation of mRNA of proteins indicative of matrix formation (Col I) and then by indicators of bone mineralization (OC). Because of these data, a study focusing on clarifying the response in cancellous bone between two and 12 hours following a 9d suspension is planned; this experiment will also investigate message levels of the proto-oncogenes.

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 24hr . 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-ß, 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.

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Morey-Holton, E.R., M.C. van der Meulen, R.T. Whalen, and S.B. Arnaud "The skeleton and its adaptation to gravity. In: Handbook of Physiology: Environmental Physiology, Part III: "The Gravitational Environment", Section 1: "Microgravity", Chapter 31." Edited by: C.M. Blatteis and M.J. Fregly. New York City, NY, Oxford University Press, pp 691-719, in press.

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Gravity-Induced Changes in Gene Expression in Arabidopsis

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Funding:

Project Identification:

Solicitation: 93-OLMSA-05

Initial Funding Date: 11/95

Expiration: 12/96

FY 1995 Funding: $99,806

Students Funded Under Research: 4

Flight Information:

Flight Assignment: BRIC-07 (STS-77, 1996)

Responsible NASA Center: Kennedy Space Center

Task Description:

The investigation proposed will attempt to identify the genes involved in gravity signal perception and transduction. The objectives include: to study changes in translatable mRNA, to isolate genes that are regulated by gravity, and to analyze the expression of genes which have known roles in calcium-mediated signal transduction pathways.

Gravity plays an important role in the normal growth and development of plants, especially in orienting roots and shoots. However, the molecular mechanisms by which plants sense and transduce the gravity signal are poorly understood. A study to investigate the effects of reduced gravity on gene expression has been initiated using *Arabidopsis* as a model system. This study involves growing *Arabidopsis* seedlings on Earth and in a canister on the Shuttle as a Biological Research in a Canister (BRIC) experiment and analysis of gravity-induced changes in gene expression. RNA isolated from seedlings grown under terrestrial gravity and in microgravity will be used for differential display to detect gravity-regulated changes in gene expression and to isolate the affected genes. To ensure an efficient BRIC experiment, a series of ground-based studies are being conducted. These studies are designed to determine: 1) the ideal seed density to obtain enough plant tissue from a single canister; 2) optimal germination surface for tissue recovery after freezing in liquid nitrogen; 3) yield and quality of mRNA from small amounts of tissue; 4) time point to freeze the seedlings; and 5) changes in gene expression that may be caused by stresses such as hypergravity and vibrations produced during launch. Ground-based studies indicate that up to 10,000 seeds could be germinated on a 100 mm diameter Petri plate. It was found that the nylon membrane is the best surface for the recovery of plant material after freezing. Results from tissue recovery studies indicate that about 20 to 40 g of tissue can be obtained

from Petri plates that fit in a single canister depending on the seedlings stage at the time of freezing. Fifty different RNA isolations using 0.2 to 0.5 g of seedlings indicate that 0.5 mg of total RNA can be obtained per gram of tissue indicating the amount of RNA that would be obtained from a single canister will be sufficient to carry out the proposed differential display experiments. Reverse transcription polymerase chain reaction experiments with the isolated RNA suggest that the RNA from the seedling is of good quality and suitable of RT-PCR and differential display. The results of the ground-based studies have not only provided important results that are used in designing the forthcoming BRIC experiments but also indicate the feasibility of the experiment. The BRIC experiment should help better understand the effects of gravity of gene regulation and provide insights into the molecular mechanism(s) by which gravity influences aspects of plant growth.

These studies will help us understand how plants perceive and respond to gravity signals.

Publications, Presentations, and Other Accomplishments:

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Bed Rest Study (ground based for LMS)

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Dr. Robert Fitts, Ph.D.	Marquette University

Funding:

Project Identification: 199-97-62-16 and 106-30-32 Solicitation: AO-OSSA-84

Initial Funding Date: 1/95 Expiration: 9/95

FY 1995 Funding: $600,000 Students Funded Under Research: 7

Joint Participation: ESA

Flight Information:

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

Responsible NASA Center: Ames Research Center

Task Description:

LMS will be the first Spacelab mission involving human research experiments to perform a ground-based integrative science study before the flight. The study will be performed in NASA's Human Research Facility at Ames Research Center, Moffett Field, California, to provide a simulation of the human life science experiments. Human volunteers will participate in the bed rest studies. This simulation of space flight mimics the effects of microgravity on the Shuttle/Spacelab crews; for example, there are similar fluid shifts and loss of muscle, bone size and strength. The ground based study has the added benefit of optimal environmental controls and provide twice the number of research subjects.

The volunteers lie on inclined beds with their feet elevated 6° above their heads, a position that causes changes to many organ systems similar to the physiologic changes experienced by astronauts during space flight. During a control period of 2 weeks, subjects will provide pre- bed rest baseline data. Following the timeline of the mission, they will then have 17 days of bed rest, followed by an ambulatory 2-week recovery period. Subjects of the bed rest study will participate in 12 of the

experiments to be performed on the LMS crew during the mission. By performing these investigations together on the ground, researchers hope to identify how one experiment is affected by another experiment. In addition, results of the study may have direct applications to people who become more inactive here on Earth.

The ground-based pilot study has been completed. Dates of performance were June 30, 1995 - August 28, 1996. The main results were the development of a workable schedule for 4 muscle testing experiments, revisions in 3 experiments planned for flight, the acquisition of reportable data in 11 experiments planned for the mission and identification of interactive effects of 2 experiments on 2 others. Information conveyed by bed rest subjects to astronauts is expected to facilitate the experiments in flight.

The information gathered during the pilot study for the LMS mission focused on musculoskeletal function, energy metabolism, circadian rhythms and central nervous system performance (mentation as well as neuromuscular). The research studies are expected to yield new information in all 3 areas which have relevance to normal people with inactive lifestyles and/or patients with disease-mandating bed rest. To date the importance of fluid balance to energy metabolism and muscle wasting is presented with new information.

Publications, Presentations, and Other Accomplishments:

Grichko, V.P. and Fitts, R.H. "Effect of 17 days of bedrest on the enzyme and metabolite profile of the slow type I fiber." Med. Sci. Sports Exerc., 1995.

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Direct Measurement of the Initial Bone Response to Spaceflight in Humans

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Funding:

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FY 1995 Funding: $103,878

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The skeleton constantly is 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, even during a short-duration 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 currently 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 in-flight response to the short-term exposure to space.

At each meal from 10 days before the mission to 7 days after, crew members will ingest an oral tracer, a nonradioactive (stable) isotope of calcium, to distinguish calcium coming from the diet from that being resorbed from bone. Measuring the isotope ratios of calcium in blood, urine, and fecal samples taken before, during, and after the mission will allow investigators to determine directly the change induced by space flight in the calcium coming from bone. They also will be able to determine how each individual adapts to this inflight change in bone resorption. All food, drink, and drug intake will be logged. This experiment will be the first to study metabolic balance in space since the Skylab studies of 1973-74.

During FY95, a ground-based bed rest simulation study will be done according to the flight protocol (as much as possible), to provide data on the interaction of the various experiments manifested for flight and to provide a ground-base set of reference data with which to compare the flight results.

A major objective of this project is to determine early (4 hour) changes in the calcium homeostatic system in space flight. This requires blood and urine sampling early in flight, including processing and freezing of blood samples. As the primary experiment on LMS using blood samples, we tested a new blood sampling system to determine if changing the system used on SLS-1 and SLS-2 would affect our results and ability to compare data with the earlier flight data. We conducted a large number of tests using previously-qualified Corvac flight tubes (used for SLS-1/2) and the blood sampling system proposed for LMS (a plastic collection system manufactured by Terumo). We also did a number of tests with the new thermoelectric cooling/freezing module to be flown on LMS as a replacement for the LSLE refrigerator used on SLS-1/2 (LSLE R/F being flown in freezer mode on LMS).

In addition to flight-specific hardware technical issues, a number of technical issues related to the measurements to be made were also addressed. We were able to qualify a new assay for bone-specific enzymes to provide additional scientific return from the serum samples we already plan to obtain from the flight with no reduction in science return from our other measurements. This was done by qualifying a new assay for 1,25-dihydroxyvitamin-D using a lesser amount of serum. These assay tests were done originally on test samples from normal subjects in the lab, and then were used for analysis of the samples from the bed rest study as a prelude to the flight. We also identified a specific interference in one of our assays done for SLS-2, where an agent (PAH) given for the renal studies produced excessive excretion of deoxypyridinoline, a bone collagen breakdown product which we use to quantify bone resorption. For LMS, this agent will not be used so there will not be any interference, but we were able to adjust our SLS-2 data to compensate for this interference and increase the confidence in our results from this flight.

During FY95, a bed rest simulation of the LMS mission was conducted at Ames Research Center, with all LMS investigators participating. While the protocol did not reflect exactly the flight timeline, it provided a good simulation of the procedures and interactions to be expected in flight. The preliminary results of this bed rest study are incorporated in a report sent to ARC.

The preliminary results obtained from the 17-day bed rest study indicate that the parameters chosen to be measured during the LMS mission show changes even during a ground-based simulation, with the ground-based results showing a comparatively smaller response than what would be expected in space flight. There are still some differences noted between bed rest and previous space flight results, clearly of a quantitative nature and perhaps of a qualitative nature as well. However, using the anticipated flight timeline for this bed rest study did not uncover any interactions with other experiments which would be expected to significantly influence our results and therefore compromise the outcome of our experiment.

The study we propose to do for LMS 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 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, its possible wide application not only in osteoporosis but in oncology, hematology and other fields may make it a fruitful area of investigation in the future.

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

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Funding:

Project Identification: E036

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Students Funded Under Research: 15

Flight Information:

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

Responsible NASA Center: Johnson Space Center

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 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 5 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 electrical activity (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.

E036 experiment was designed to determine the relationship between hormonal levels, global electromyographic (EMG) activity of a muscle and motor in healthy men before, during and after 17 days of bed rest. In the present paper, the authors looked more specifically at how muscle unloading during bed rest respectively influenced 1) muscle activation, 2) motor control, and 3) growth hormone release.

Effect of bed rest on 24-hour EMG activity
It was previously reported in animal studies that unloading with hindlimb suspension resulted in a short term reduction in the EMG activity of the ankle extensors, the soleus (SO) and gastrocnemius medialis (GM), and an increase in activity of their antagonist muscle, the tibialis anterior (TA). Moreover, the recruitment pattern of the SO and GM were altered during unloaded condition. In normal load bearing activity, the SO is recruited during both postural and low level activity whereas the GM muscle is activated mainly during activities requiring higher force-velocity outputs. During unloading, activity in GM is seen in the absence of soleus activity. The objectives of the 24 hour EMG recording were thus 1) to assess the level of activation of postural and non-postural muscles during normal activity and during prolonged bed rest, and 2) to investigate the recruitment pattern of slow and fast ankle extensors. EMG activity of the SO, GM, TA, Biceps Brachii (BB), and Triceps Brachii (TB) muscles were recorded for 24 hour periods pre, during and post 17 days of 6° incline bed rest.

The experiment was divided into a lower limb portion, examining torque and EMG measurements from ankle dorsiflexors and plantarflexor muscles, and an upperlimb portion examining torque and EMG measurements from elbow flexor and extensor muscles. Muscle contractions were isometric for all measurements reported in the present study. In the four functional muscle groups studied (plantarflexors, dorsiflexors, elbow flexors, and extensors), no significant effect of bed rest on the perception of muscle output was found. No significant change in the slope or y-intercept, nor in the correlation coefficient, of this relationship was found in a comparison of bed rest and control/recovery testing. It was concluded that perception of muscle output was not affected by bed rest in this study group. A somewhat surprising finding in this study was the remarkable acuity, in all subjects, of muscle output perception across the entire muscle output range. These findings emphasize the important question of whether or not the high level of muscle output discrimination would be lost or altered in microgravity.

Changes in hormone response to muscle activity during bed rest
The fatigue test involved a series of unilateral isometric plantar flexions and included 4 maximal voluntary contractions (MVC), 48 contractions at 30% MVC, and 12 contractions at 80% MVC all performed at a 4:1 s work:rest interval. Additional motor control testing preceded the fatigue test.

Blood was collected for hormonal analysis prior to motor control tests and immediately following the fatigue test. Growth hormone (GH) measured by radioimmunoassay was unaffected by the fatigue test during all testing periods. However, a hypophysectomized rat GH bioassay of tibial cartilage growth indicated a significant increase ($p<0.05$) of tibial growth factor (TiGF; $\mu g \cdot L^{-1}$) following the fatigue test (Con1 2146 ± 192 to 3565 ± 197; Con2 2162 ± 159 to 4161 ± 204; Fig. 4). This TiGF response was absent at BR1 and significantly decreased ($p<0.05$) by BR2 (2433 ± 185 to 2105 ± 106) and BR3 (2594 ± 211 to 2085 ± 109). By Rec3, the TiGF response had returned (1881 ± 75 to 4160 ± 315). Testosterone and thyroid hormones (T3 and T4) were unchanged during all testing periods. In conclusion, the release of TiGF in response to a fatigue test was inhibited during bed rest, but had returned by 10-11 days of recovery.

This project has 4 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 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.

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 seems to become less efficacious with bed rest and we hypothesize that similar effects will be caused by chronic exposure to space flight.

Each phase of these experiments have 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 excepted 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.

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Effect of Weightlessness on Human Single Muscle Fiber Function

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Funding:

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FY 1995 Funding: $200,000

Students Funded Under Research: 4

Flight Information:

Flight Assignment: LMS, (STS-78, 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 (VO_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, in-flight, 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.

In FY95, we conducted two support studies: 1. A bed rest study designed to mimic the test sequence of the LMS flight,. and 2. A unilateral lower leg suspension study in which we demonstrated the need to prevent post-flight activity (walking or any type of exercise) before obtaining the muscle biopsies. The main results of these studies are summarized here.

Bed Rest Study.

1. The slow type I fibers showed a small (5%) but significant decline in fiber diameter.
2. There was no significant change in the fiber type distribution in the soleus.
3. Fiber force showed a small but significant decline which was explained by the reduced fiber size.
4. Fiber stiffness declined while the force/stiffness ratio increased. This suggests that the spacing between the filaments increased as a result of selective loss of contractile proteins. The EM results support this conclusion.
5. The maximal shortening velocity increased and, since there was no change in the myosin heavy chain, we conclude that the velocity increase was caused by the increased filament spacing, and by an increased expression of the myosin light chain 3.
6. Peak power significantly declined and this can be attributed to the reduced force as the velocity at any given load increased.
7. The enzymes involved with glycogen synthesis and metabolism increased.
8. Fiber glycogen and lactate increased. The increased glycogen was observed in all subjects and the biochemical analysis was confirmed by EM which showed increased glycogen particles.

Unilateral Lower Leg Suspension (ULLS) Study.

It has been proposed that ULLS may be an appropriate ground-based model with which to study non-weight bearing induced alterations in human muscle function. We utilized this model to determine whether or not post-flight reloading could be expected to induce muscle damage. If damage was observed, this would make a strong case for obtaining the post-flight muscle biopsies before reloading, walking or performing any type of exercise. We observed the following results.

1. ULLS induced significant decreases in the type I fiber diameter and the peak isometric force.
2. The type I fiber peak tension (force/CSA) remained unchanged.
3. In contrast to bed rest, the ULLS did not cause an increase in the type I fiber maximal shortening velocity (V_o).
4. The peak power of the slow type I fiber was reduced by 30%, and this change was entirely due to the reduced force generating capacity of the fiber.
5. The ULLS caused some disorganization of the myofibril structure, and following 6 hours of reloading the disruption increased and many fibers showed cellular debris indicating fiber damage.
6. These results document the necessity to prevent post-flight reloading before obtaining the post-flight biopsy.

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 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. 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 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.

Publications, Presentations, and Other Accomplishments:

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Widrick, J.J., Romatowski, J.G., Blaser, C.A., Norenberg, K., Costill, D.L., and Fitts, R.H. "Peak force and maximal shortening velocity of soleus muscle fibers after 17 days of bed rest." Med. Sci. Sports Exerc., (In Press).

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Magnetic Resonance Imaging after Exposure to Microgravity

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Project Identification: E029

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FY 1995 Funding: $70,131

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

After the 8-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 also will 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 2 weeks in space. These findings may indicate alterations in the ability of the bone marrow to produce new red blood cells.

1. Completed a bed rest study at Ames Research Center designed to mimic the LMS flight. The DEXA whole body data analysis has been completed. The MRI muscle volumes for the calf and spectroscopy of the spine are complete.

2. We have contracted with a vendor to provide a portable MRI unit at the landing sites. The necessary coordination with KSC and Dryden is ongoing.

3. The Hologic company has been contacted to provide DEXA devices for the landing sites. At this time no firm commitment has been obtained. A back-up plan has been formulated.

4. The amount of fluid shift to be expected in the lower limbs during an MRI scanning protocol and after overnight rest was determined.

5. The technique to measure fat-water ratio was developed using *in vitro* phantoms. The short and long-term *in vivo* precision is proceeding at this time.

6. The final preparations for the LMS flight are proceeding. Two of the crew were tested to determine if metal implants would interfere with the MRI testing; results showed that they would not.

Space flight measurements have documented that significant bone and muscle atrophy occurs during weightlessness. Knowledge of the extent and temporal relationships of the 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 one 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 often involves the intervertebral discs. Bed rest 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 bed rest 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.

Publications, Presentations, and Other Accomplishments:

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Lignin Formation and Effects of Microgravity: a New Apporach

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Funding:

Project Identification:

Solicitation: 93 OLMSA-07

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FY 1995 Funding: $79,690

Students Funded Under Research: 3

Flight Information:

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

Responsible NASA Center: Kennedy Space Center

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 it has an adverse effect on wood quality and texture.

Conifer seedlings will be placed in the Plant Growth Facility (PGF) in an orientation that favors reaction wood formation in Earth's gravity. The crew will perform a daily status check of PGF 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 compliment 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.

Bending experiments have been conducted in order to determine the onset of reaction wood formation. It has been shown that a 24 hour-bending period is sufficient to induce reaction wood formation on Earth. These experiments have been carried out for both loblolly pine and Douglas fir. Protocols have

been developed for harvesting the tissue. The fixative bags have been tested. Preliminary work regarding the metabolic flux of phenylpropanoid monomers into lignin has been investigated, i.e., HPLC conditions have been established for the separation of all the acids, CoA esters, aldehydes and alcohols of the phenylpropanoid pathway.

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 both lumber and 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.

Publications, Presentations, and Other Accomplishments:

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Human Sleep, Circadian Rhythms and Performance in Space

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Funding:

Project Identification: E948

Solicitation: NRA

Initial Funding Date: 8/90

Expiration: 6/97

FY 1995 Funding: $268,618

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

This is the first simultaneous study of sleep, circadian rhythms, and task performance of a group of astronauts in response to a microgravity environment. The experiment will evaluate effects caused by microgravity and by the absence of terrestrial time cues (zeitgebers) and normal social contacts. Scientists hypothesize that the severe weakening of social and physical zeitgebers during the mission and/or unusual conditions within the environment (microgravity, cramped conditions, and stress) will disturb circadian rhythms which, in turn, will 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 will wear a special belt pack connected to a sleep cap with 10 electrodes attached to the head. The system will provide data about brain waves (electroencephalography), eye movements (electro-oculography), and muscle tone (electromyography) while the crew member is sleeping. These data allow scientists to categorize each minute of sleep by various types and depths. During the 72 hours, another belt pack recorder receives a signal from a temperature sensor indicating the crew member's core body temperature every 6 minutes. Circadian rhythms also will be evaluated by measuring urine electrolyte and hormone concentrations at each voiding, by mood and activation testing every 2 hours during the wake cycle, and by performance testing before each meal. Crew members will keep a diary to record sleep quality and alertness on awakening and will answer 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 will use the Payload and General Support Computer.

Data will be compared with pre- and post-flight tests on Earth. Also, an identical ground study will be performed after the mission under the direction of Dr. Alexander Gundel of the Institute of Aerospace Medicine in Cologne, Germany.

FY 1995 saw significant progress for this experiment on two different fronts. With regard to the LMS flight itself, a number of equipment changes were implemented with respect to the computer used, and to the core body temperature collection device, as well as to the addition of wrist actigraphy. These changes required a rewrite of much documentation and a change in our experiment software. Crew orientation training took place in Pittsburgh and was deemed highly successful. Task training took place in Houston, and resulted in some useful suggestions for software and hardware changes by the astronauts. These have now been implemented and have undoubtedly strengthened the experiment.

On a second front, the SACS experiment was heavily involved in the LMS bed rest study at NASA Ames which took place in the summer of 1995. Substantial efforts were required to develop procedures, to purchase equipment, to prepare the materials and to run the study. Thanks to the heroic efforts of the SACS Pittsburgh team and the compliance of the eight bed rest subjects, very useful and informative data were obtained, related both to the execution of the study in space and to the scientific question of how bed rest and the absence of sunlight might affect circadian rhythms, sleep and performance.

Life on Earth has developed to be in tune with the 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 24h, but is faster or slower than that figure. For example, for humans, the figure is about 24.3 -25.0h, 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 24h 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.

Publications, Presentations, and Other Accomplishments:

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Canal and Otolith Integration Studies (COIS)

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Funding:

Project Identification:

Solicitation: 93-OLMSA-07

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The research protocols in the Canal and Otolith Integration Studies (COIS) 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. (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.

This experiment is only a few months away from flight. Flight hardware has been delivered and integrated into the LMS spacelab module, crew training is at an advanced phase, and baseline data collection will begin in one month.

As a part of the preparation for this experiment on the LMS flight, we participated in a bed rest study to investigate both bed rest as a stimulus and the interaction of our experiment with other life science experiments currently in the timeline. Data analysis from this bed rest study has been completed and a report is currently being drafted.

This experiment is a follow-on set of studies first performed as a part of the Microgravity Vestibular Investigations (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 modify completely 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 insure the safety, health and well being of future astronauts.

Publications, Presentations, and Other Accomplishments:

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Microgravity Effects on Standardized Cognitive Performance Measures

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Funding:

Project Identification: E963

Solicitation: 89-OSSA-13

Initial Funding Date: 11/89

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FY 1995 Funding: $245,426

Students Funded Under Research: 5

Joint Participation: DoD

Flight Information:

Flight Assignment: LMS, (STS-78)

Responsible NASA Center: Johnson Space Center

Task Description:

A cooperative USAF/NASA experiment was flown as part of the payload for the Second International Microgravity Laboratory on-board the Space Shuttle Columbia (STS-65) in July 1994. The experiment studied the interactive effects of microgravity and fatigue on the cognitive functioning of three astronauts for 13 days on a dual-shift mission. A Performance Assessment Workstation (PAWS) was developed and validated for space flight to collect cognitive performance test data. The performance tests were selected from the DOD Unified Tri-Service Cognitive Performance Assessment Battery (UTC-PAB). The tests measured short-term memory, spatial processing, attention, tracking, and dual task timesharing. All three astronauts completed 40, 20-minute sessions of a battery containing 6 cognitive performance tests and 2 subjective scales (mood and fatigue) on a laptop computer. Twenty-four sessions were preflight, 13 sessions were in-orbit, and 3 sessions were postflight. It was demonstrated that cognitive and psychomotor performance can be reliably measured in the microgravity environment. The performance measures were sensitive to the combined stressor effects of the microgravity environment. In general, performance patterns of the astronauts in-orbit and during ground-based periods were comparable when learning of the task had stabilized with the exception of Memory Search for one subject and Unstable Tracking performance in another subject. Both of these performance decrements were related to fatigue. Single-subject mathematical models of astronaut preflight performance revealed that predicted learning levels for two subjects were not achieved in tracking, short-term memory, and directed attention. Even if one considers the converging evidence

from an extremely limited number of other in-flight experiments, the currently available database about human performance in space is too small to warrant final conclusions about cognition and visuomotor performance while living and working in microgravity. Isolation of microgravity as the single stressor causing in-orbit performance deterioration cannot be fully determined from these results. Therefore, additional ground-based control research studies and more in-orbit subjects are required before these results can be generalized to future space travelers. PAWS is currently manifest on STS-78 for the Life and Microgravity Sciences mission and will provide this experiment with four additional subjects.

Prior to and during FY95, a Performance Assessment Workstation (PAWS) was developed and validated for space flight to collect cognitive performance test data. The performance tests were selected from the DOD Unified Tri-Service Cognitive Performance Assessment Battery (UTC-PAB). The tests measured short-term memory, spatial processing, attention, tracking, and dual task timesharing. Three astronauts have completed 40, 20-minute sessions of a battery containing 6 cognitive performance tests and 2 subjective scales (mood and fatigue) on a laptop computer. Twenty-four sessions were preflight, 13 sessions were in-orbit aboard STS-65, and 3 sessions were postflight. It was demonstrated that cognitive and psychomotor performance can be reliably measured in the microgravity environment. The performance measures were sensitive to the combined stressor effects of the microgravity environment.

In general, performance patterns of the astronauts in-orbit and during ground-based periods were comparable when learning of the task had stabilized with the exception of Memory Search for one subject and Unstable Tracking performance in another subject. Both of these performance decrements were related to fatigue. Single-subject mathematical models of astronaut preflight performance revealed that predicted learning levels for two subjects were not achieved in tracking, short-term memory, and directed attention.

Even if one considers the converging evidence from an extremely limited number of other in-flight experiments, the currently available database about human performance in space is too small to warrant final conclusions about cognition and visuomotor performance while living and working in microgravity. Isolation of microgravity as the single stressor causing in-orbit performance deterioration cannot be fully determined from these results. Therefore, additional ground-based control research studies and more in-orbit subjects are required before these results can be generalized to future space travelers.

PAWS is currently manifest on STS-78 for the Life and Microgravity Sciences (LMS) mission and will provide this experiment with four additional subjects. This year's work has integrated the PAWS experiment with the training, in-orbit and recovery timelines of the other LMS experiments. A bed rest study conducted at NASA/Ames Research Center revealed that the PAWS experiment was compatible with the requirements of the other life sciences experiments. Minor programming changes to the PAWS software will provide functional compatibility with another LMS experiment and facilitate data sharing and interpretation of results.

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, counter-measures to the stressor/s 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 muscle and bone tissue, space travelers are required to use their brain to accomplish tasks similar to Earth dwellers. Therefore, performance degradation 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 common man on Earth to identify and counter the conditions responsible for reduced productivity and job satisfaction and increases of 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 subject judgments that are somewhat independent of productivity.

Publications, Presentations, and Other Accomplishments:

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Schiflett, S.G. "Cognitive performance assessment during sustained operations in unique environments." 103rd Convention American Psychological Association, New York, NY, August 11-15, 1995.

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

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Project Identification: E871

Solicitation: AO-OSSA-84

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FY 1995 Funding: $129,802

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

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 impaired performance, increased susceptibility to disease, and delayed healing of wounds. Such a loss during space flight may affect in-flight performance and impair the ability of the individual to function adequately during the critical phases of re-entry and landing.

The doubly labeled water method is a highly accurate means of measuring energy output 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 ($^{18}0$) is ingested by the payload crew. The two isotopes leave the body at different rates. Deuterium leaves primarily in urine while 180 leaves in both water and exhaled carbon dioxide (CO_2). The difference in loss rates is equal to the rate of CO2 production, which is directly related to the rate of energy expenditure. Crew members will provide samples of urine and saliva and will collect galley water to correct for any background changes in the drinking water. Also, they will monitor their dietary and drug intake, keep a daily activity log, and measure their body mass with the Space Linear Acceleration Mass Measuring Device (SLAMMD). These measurements

will be taken during 2 consecutive 6-day blocks of time before, during, and after the mission, a total of 36 days. Energy balance will be 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 in-flight data against the combination of the preflight and matched bed rest data will indicate whether the energy costs of living and working in space are greater or less than those on Earth for comparable activity.

During FY 1995, a 17-day bed rest study with 6° head down tilt was conducted in the Clinical research Center of the NASA-Ames Research Center. 8 healthy adult males were recruited from the local community. The study was divided into three phases, a 15-day pre-bed rest ambulatory period followed by 17 days of bed rest and ending with a fifteen day recovery period. During the 47 days of the study the subjects received all their nutrition from the research center. Six subjects were dosed with $^{2}H_{2}{}^{18}0$ for the energy expenditure measurements. Two randomly chosen subjects, were not given any isotopes were used to measure, and hence to correct for any variations in background water isotopic enrichments that occurred during the study period. Saliva was used to sample the body water pool.

This additional important ground based data will permit the comparison of the inflight data against a ground based bed rest study which included an a series of activities and physiological measurements designed to match the LMS inflight measurements.

Analyses are still in progress. However some important data was gained concerning when to sample body water post-isotope dosing inflight. The original protocol called for sampling body water 6 and 8 hours post-dosing during bed rest. In healthy ambulatory subjects only 3-4 hours are required for isotopic equilibration. Nevertheless, prior to this experiment we had some suspicions that isotopic equilibration for the determination of body water might take longer in space than on the ground. This supposition was based on the TBW data from prior experiments. The results of the bed rest study showed that sampling at 6 and 8 hr. overestimated body water by about 1% due to incomplete equilibration by about 1% ($p<0.07$) which will translate into an over-estimate of the energy expenditure rate by 1%. Accordingly, we have requested that NASA change the proposed inflight timeline for the LMS study so as increase the time elapsed between dosing and saliva collection.

This project will eventually provide information on the relationship between muscle loss, energy expenditure and activity. While the space flight related muscle 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.

Extended Studies of Pulmonary Function in Weightlessness

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Funding:

Project Identification: E030

Solicitation: AO-OSSA-84

Initial Funding Date: 7/92

Expiration: 6/97

FY 1995 Funding: $1,165,000

Students Funded Under Research: 2

Flight Information:

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

Responsible NASA Center: Johnson Space Center

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 4 times before the flight, several times during flight, and 5 times in the 2 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 Respitrace 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.

Crew training is ongoing. All hardware is delivered to KSC and has completed level IV integration. Baseline data collection is about to start which will represent the first orbital data collection. The integrated bed rest study performed at NASA/Ames in the summer of 1995 is complete. Data analysis is mostly complete.

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 bed rest study will provide a useful set of data on the effect of long-term bed rest 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.

Publications, Presentations, and Other Accomplishments:

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Prisk, G.K., Elliott, A.R., Guy, H.J.B., Kosonen, J.M., and West, J.B. "Pulmonary gas exchange and its determinants during sustained microgravity on Spacelabs SLS-1 and SLS-2." J. Appl. Physiol., 79, 1290-1298 (1995).

Prisk, G.K, Guy, H.J.B., Elliott, A.R., and West, J.B. "Inhomogeneity of pulmonary perfusion during sustained microgravity on Spacelab SLS-1." J. Appl. Physiol., 76, 1730-1738 (1994).

Prisk, G.K., Guy, H.J.B., Elliott, A.R., Paiva, M., and West, J.B. "Ventilatory inhomogeneity determined from multiple-breath washouts during sustained microgravity on Spacelab SLS-1." J. Appl. Physiol., 78, 597-607 (1995).

Development of the Fish Medaka *in Microgravity*

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Project Identification:

Solicitation: Unsolicited

Initial Funding Date: 1/95

Expiration: 12/97

FY 1995 Funding: $

Students Funded Under Research: 5

Flight Information:

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

Responsible NASA Center: Ames Research Center

Task Description:

The Life and Microgravity Spacelab (LMS) Space Tissue Loss-B (STL-B) hardware will be 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.

About two hours after fertilization, approximately six *Medaka* embryos will be isolated into each of six optical chambers of the STL-B hardware. The embryos will be cooled to 12°C, which will slow embryo development during preflight processing. Soon after the Shuttle achieves orbit, the temperature will be increased, allowing development to continue. At predetermined intervals during the mission, video microscopy will be downlinked to researchers on the ground. These downlinks are planned to observe hey phases of *Medaka* development. Also, at specified intervals, embryos will be fixed for postflight evaluation.

One of the reasons for selecting the fish *Medaka* as an ideal model for studying vertebrate development in space is its ability to tolerate reduced temperatures during early embryogenesis. This has permitted us to slow down early embryogenesis until the embryos would be exposed to microgravity. A series of

temperature shift trials have been run to determine the appropriate temperatures in which to hold *Medaka* embryos so that a minimal amount of development has occurred prior to arrival in microgravity. The length of time the embryos are held in a slow developing state depends on the flight hardware turnover time established for each particular flight.

Medaka fish embryos are also optically clear, allowing direct observation of embryonic development by video-microscopy. As noted above, 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 two separate occasions. The first, STS-59, was considered a hardware flight test. *Medaka* embryos were flown on this mission and all systems checked out in terms of biocompatibility. In addition, we were able to obtain good video images of the developing embryos during space flight. The second flight, STS-70, provided the opportunity for another series of video observations and histological examinations on the effects microgravity might have on the development of the *Medaka*. These analyses are currently underway.

One of the direct molecular genetic analyses we have undertaken has been cloning of the *Medaka* homeobox-containing gene Hoxa-4. A genomic clone of Hoxa-4 was isolated from a *Medaka* genomic library, sequenced in entirety, and the putative promoter and coding regions deduced by their homology with the mouse and chicken Hoxa-4 genes. We plan to use the *Medaka* Hoxa-4 gene as a marker of embryonic development for analyzing the effect of microgravity stress on embryonic segmentation. To this end, we are currently determining the expression pattern of *Medaka* Hoxa-4 during embryogenesis, under normal conditions. Our preliminary Northern blot analysis of total RNA isolated from embryos pooled at various stages of development reveals the expression of three transcripts of 6.5, 2.4 and 1.6 kb, first detected at stage 21, when the *Medaka* embryos have six to eight somites. The next series of experiments will examine embryos at various stages of development by *in situ* hybridization. Experiments are underway to determine the optimal fixation-embedding procedures for handling the embryos at various stages. Our sequence analysis of the *Medaka* Hoxa-4 gene showed several sequences conserved in mouse and chicken, suggesting a role in the regulation of Hoxa-4 pattern of expression. These conserved regions, involving both promoter and intron regions (in addition to the coding regions) will be used to drive expression of reporter constructs in a cross-species manner. Our initial analysis has focused on the introduction of mouse sequences into the *Drosophila* and examining the expression of the reporter constructs in embryos and imaginal disks. We plan to extend this analysis by generating transgenic *Medaka*.

As noted above, we have been able to conduct initial experiments examining the effect of microgravity on Medaka development on the STS-70 space shuttle flight, in July 1995. Video data from the previous flight experiments have provided information on the rates and intensities of surface contraction waves over the yolky portion of the early embryo. These contraction waves appear to be quite strong and at the upper range of the normal range for embryos grown in 1-g environments. We are beginning a detailed analysis of this phenomenon. On STS-70, two sets of 6 embryos were not fixed and returned to Earth, to Dr. Wolgemuth's laboratory, together with the 6 embryos kept at KSC as ground controls. The 18 embryos were maintained at 21-25°C under a 16/8 hours light/dark cycle and allowed to hatch. The fry were transferred to larger tanks and grown. The first eggs were observed on November 10th in the group #1 fish. We have now combined groups #1 and #2 to increase the chance of mating. Our analysis to date with regard to swimming and mating behaviors and to body growth, suggest that the young adults developed from embryos developing in microgravity appear normal. We will now assess the developmental outcome of their progeny.

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 level, 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.

Publications, Presentations, and Other Accomplishments:

Crotty, D.A., A.I. Packer, T. Haerry, W.J. Gehring and D.J. Wolgemuth "Elements involved in the in vivo regulation of Hoxa-4 expression." EMBO Workshop on The Homeobox Genes in Development and Evolution, Session VI, Ascona, Switzerland, 1995.

Wolgemuth, D.J. and A. K. Murashov "Models and molecular approaches to assessing the effects of the Microgravity environment on vertebrate development." Amer. Soc. for Gravitational and Space Biol., Bulletin 8, 63-71 (1995).

Yost, H.J., C.R. Phillips, J.L. Boore, J. Bertman, B. Whalon and M.V. Danilchik "Relocation of mitochondria to the prospective dorsal masrginal zone during Xenopus embryogenesis." Devel. Biol., vol 170, 83-90 (1995).

Role of Corticosteroids in Bone Loss during Space Flight

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Funding:

Project Identification:

Solicitation: AO-OSSA-84

Initial Funding Date: 9/93

Expiration: 8/97

FY 1995 Funding: $101,848

Students Funded Under Research: 1

Flight Information:

Flight Assignment: LMS (STS-78, 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 both 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.

The flight experiment is designed to determine whether maintaining serum corticosteroids at normal levels will affect the bone changes that occur in the weightlessness of space. As a prerequisite for this experiment, serum corticosteroids must be manipulated in rats by a combination of adrenalectomy and supplementation with normal, physiologic levels of corticosteroids. Therefore, the focus of the supporting ground-based studies was to develop expertise in this surgical procedure and to determine the proper dose of corticosteroids for attaining physiologic serum levels of the hormones. The success of adrenalectomy (ADX) was confirmed by radioimmunoassay which revealed undetectable levels of serum corticosteroids in nearly all ADX rats. The first attempts to supplement ADX rats with physiologic levels of serum corticosteroids with Alzet osmotic minipumps commercially available pellets were unsatisfactory. These minipumps and pellets failed to release their hormonal contents at a constant rate. We then synthesized our own pellets by dissolving corticosterone and aldosterone in cholesterol. In a dose response study, it was determined that implanting ADX rats with pellets containing 35 mg of corticosterone dissolved in 100 mg of cholesterol resulted in nearly constant, physiologic serum levels of corticosterone (~50 ng/ml) for a 28 day period. Histomorphometric analyses revealed that the bones from these animals were not different from those of sham operated control rats. Therefore, the objectives of the supporting, ground-based studies have been largely fulfilled. More specifically, the investigative team has developed expertise in adrenalectomy and the synthesis of corticosterone pellets that maintain normal serum levels of the hormone when implanted in ADX rats.

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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Funding:

Project Identification:

Solicitation: 93-OLMSA-06

Initial Funding Date: 7/95

Expiration: 6/97

FY 1995 Funding: $67,867

Students Funded Under Research: 1

Flight Information:

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. Bird and human 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.

A working relationship between the Russian and the American scientists was established. We have now worked together in a collaborative effort to dissect quail embryos at different stages of development. Using tissues primarily from the ground-based controls, our laboratory has successfully developed and applied an approach to purify messenger RNA from fixed cardiac and pectoral skeletal muscle. The protocols for reverse transcription-polymerase chain reaction (RT-PCR) are now being established that will be used to identify the expression of the cardiac troponin T isoforms and the cardiac and slow skeletal muscle troponin I genes. We have successfully used primers based on the rabbit cardiac complementary DNA sequence to generate two appropriately sized RT-PCR products of the cardiac Troponin T isoforms from the fixed tissue that was dissected from the embryo by the Russian and American scientists. The continued development of these protocols will allow us to successfully determine if microgravity alters the expression of these isoforms during embryonic development.

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. Cardiac and pectoralis or wing bud tissue will be harvested from 1) flight group, eggs laid on earth and fixed in space at gestational ages of 7, 10, 14, and 17 days: 2) time delayed synchronous animal group (a control group sacrificed to mirror the flight group): and 3) laboratory control eggs. Reverse transcription-polymerase chain reaction (RT-PCR) will be used to amplify isoform-specific sequences, using primers synthesized on the basis of their published cDNA sequences. The PCR products will be cloned and sequenced to identify isoform-specific products. Since alternative RNA splicing is the basis of the troponin T isoforms and the cardiac and slow skeletal muscle troponin I genes have shared sequences, competitive reactions and single pairs of primers will be used to quantify changes in the relative amount of one isoform to another during development. Two-way analysis of variance will be used to test for the effect of microgravity. Given the similarity of the developmental programs in human and bird, the results of this study will be relevant to human development and function in space.

Publications, Presentations, and Other Accomplishments:

Anderson, P.A.W. "The Immature and the Adult Heart so alike, yet so different." Pediatric Grand Rounds, New York University Medical Center, New York, NY, May, 1995.

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Anderson, P.A.W. "no title." Cardiology Grand Rounds, Department of Medicine, Albert Einstein College of Medicine, New York, NY, August 1995.

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Anderson, P.A.W., Greig, A., Mark, TK.M., Malouf, N.N., Oakeley, A.E., Ungerleider, R.M., Allen, P.D., Kay, B.K. "Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart." Circ Res, 76, 681-686, 1995.

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Environmental Radiation Measurements on Mir Space Station

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Funding:

Project Identification: FBI 3 and FBI 4

Solicitation: 94 OLMSA-01

Initial Funding Date: 5/95

Expiration: 5/98

FY 1995 Funding: $200,000

Students Funded Under Research: 4

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

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 details information on shielding effectiveness in the 51.6 degree orbit. The systematic series of measurements made during the approach of solar minimum (Sept. 1997), will measure solar cycle effects on environmental radiation levels and include the maximum doses of galactic cosmic rays for this cycle.

Six Area Passive Dosimeter (APD) packages for deployment inside the Mir Core and Kvant 2 modules during NASA-2 have been assembled and shipped to JSC for integration in STS-76. Fabrication of the External Dosimeter Array (EDA) hardware for exposure outside Mir on NASA-3 is underway.

- Measure mission dose equivalent rates and LET spectra using passive dosimeters on NASA-2 and 3.
- Map internal radiation environment of Mir using Area Passive Dosimeters (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.

Publications, Presentations, and Other Accomplishments:

Badhwar, G.D., Atwell, W., Benton, E.V., Frank, A.L., Keegan, R.P., Dudkin, V.E., Karpov, O.N., Potapov, Y., Akapova, A.B., Magradze, N.V., Melkumyan, L.V., and Rshturi, S.B. "A study of the radiation environment aboard the Space Shuttle flight STS-57." Rad. Meas., vol. 24, no. 3, 283-289 (1995).

Adaptive Changes in Cardiovascular Control at μG

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Funding:

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Solicitation: 94 OLMSA-01

Initial Funding Date: 1/95

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FY 1995 Funding: $19,890

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA Mir Program Incr. 6 & 7 (MIR-25 & 26, NASA-6 & 7)

Responsible NASA Center: Johnson Space Center

Task Description:

The present experiment shares a common approach with our experiment "Integration of Neural Cardiovascular Control in Space," scheduled for Neurolab (1998).

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 1G. 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 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.

Detailed protocols have been developed for pre-, post-, and in-flight studies. The experiment team has visited Moscow and Star City and has had extensive discussions with Russian colleagues and space agency officials.

Instrumentation is being developed in collaboration with JSC. A parallel experiment is being planned for the German flight Mir '96 with Dr. Friedhelm Baisch, DLR, Cologne as principal investigator and the University of Texas Southwestern Medical Center group as Co-I's.

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.

Publications, Presentations, and Other Accomplishments:

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

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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 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 disorientating 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; 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 (EOG) recording methods will be used to measure eye movements. Muscle activation patterns will be determined by recording electromyographic (EMG) signals from the muscles of both legs.

This first flight opportunity for this study is Mir-21 which launches on February 21, 1996 and returns on July 25, 1996.

Summary of Progress
1) Integration of video motion analysis system with force plate, accelerometer, electromyographic (EMG) and electrooculographic (EOG) data acquisition systems (see below for details).
2) Set-up and test of hardware in Star City, Russia.
3) Preflight data collection #1, Sept. 95, Star City, Russia.
4) Preflight data collection #2, Jan. 96, Star City, Russia.

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 of the adaptation process, the development of an integrated data acquisition system will allow for the investigation of the interaction and synergies of the various sub-systems used to produce coordinated movement strategies during locomotion. Simultaneous collection of the many variables necessary to perform a 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 electromyography (EMG) from the neck, trunk, and right lower limb, 4) vertical and horizontal eye movements using DC-electrooculography (EOG), 5) heel strike and toe off using foot switches, 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, CA), 2) two triaxial accelerometers (Entran Sensors & Electronics Fairfield, NJ), 3) a seven-channel pre-amplified surface EMG amplifier system (Therapeutics Unlimited, Davenport, IA), 4) a two-channel Denver University EOG amplifier, 5) eight pressure-activated foot switches (MotionLab Systems Inc. Baton Rouge LA), 6) a Biomobile force plate (Kistler Instruments, Amherst, NY), and 7) a motor-driven treadmill (Quinton Instrument Co. Seattle, WA).

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 locomotion 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 6 cameras such that the resolution was maximized in both configurations and minimal camera movement was required to reconfigure. This setup 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 the Johnson Space Center designed to examine microgravity-induced adaptive modification of spatial orientation and motion perception processes, gaze control mechanisms, 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 aged 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 of 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 flights of longer duration, we may find changes that are not 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 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.

Publications, Presentations, and Other Accomplishments:

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Bloomberg, J.J., Huebner, W., Layne, C., McDonald, P., Reschke, M., Peters, B., and Smith, S. "Evaluation of microgravity induced adaptive modification in sensory-motor function." American Institute of Aeronautics and Astronautics, Life Sciences and Space Medicine Conference, Houston, TX, April 3-5, 1995. Abstract in AIAA Book of Abstracts, p.22-23.

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Autonomic Mechanisms During Prolonged Weightlessness

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Funding:

Project Identification: E709

Solicitation: 94 OLMSA-01

Initial Funding Date: 10/95

Expiration: 9/98

FY 1995 Funding: $ 216,666

Students Funded Under Research: 3

Joint Participation: NIH

Flight Information:

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.

During the past year, much progress has been made regarding the organizational aspects of this complicated project. Experimental integration with other members of the Autonomic Control Team is complete. Inflight hardware has been defined, and steps have been taken to test the equipment astronauts will be asked to use during flight. One of the team's primary concerns (the measurement of cerebral blood flow in space), is close to being resolved. Ground-based studies designed to address specific scientific aspects of each experimental protocol are currently underway.

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 attract 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.

Publications, Presentations, and Other Accomplishments:

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Effects of Gravity on Insect Circadian Rhythmicity

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Funding:

Project Identification: Solicitation: 94 OLMSA-01

Initial Funding Date: 11/94 Expiration: 9/97

FY 1995 Funding: $164,128 Students Funded Under Research: 1

Flight Information:

Flight Assignment: NASA-MIR-1B

Responsible NASA Center: Ames Research Center

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) 1G 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 initial phase of these studies, the investigators met to discuss and plan the details of the first flight protocols, with the assistance of personnel from NASA Ames Research Center (ARC). These discussions included definition and refinement of the hardware for flight and ground studies, the schedule of the experiments, a timetable for the project and methods to ensure coordination of activities at our three institutions (University of California Davis, Vanderbilt University & The Institute for Biomedical Problems). After obtaining USDA approval, 50 *Trigonoscelis gigas* were transported from

Moscow to the two labs of the U.S. investigators. At Vanderbilt University, using these imported beetles, Dr. Wassmer is conducting experiments examining their activity rhythms and comparing them with other Tenebrionid beetles. At the University of California, Davis, Dr. Hoban-Higgins is conducting experiments which will establish the light characteristics necessary for entrainment and phase shifting this species. Thus far, we have been able to record robust rhythms from these animals using the hardware supplied by ARC. Rhythms have been recorded in a 24-hour light/dark schedule, constant light and constant darkness. The lighting device used in these studies has been green LEDs. These were chosen for their low power requirements and the quality of the light they emit; the spectrum of the light from an LED does not change as the unit ages, unlike other light sources. These studies have been designed to support the first flight experiment which is scheduled to occur in August-December of 1996. In addition to the flight experiment, we will be conducting a delayed synchronous ground control experiment at UC Davis.

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 circadian timing system (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 had 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, 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.

Publications, Presentations, and Other Accomplishments:

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Sleep and Vestibular Adaptation

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Funding:

Project Identification: E639c

Solicitation: 94 OLMSA-01

Initial Funding Date: 7/95

Expiration: 9/98

FY 1995 Funding: $7,500

Students Funded Under Research: 4

Flight Information:

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 propose to 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.

Since beginning work in mid-July of this year, we have been proceeding through the ED and CDR phases of the project. During this time, our efforts have been devoted to finalizing hardware and software design and production.

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 workload or uncomfortable sleeping conditions of the spacecraft. It is the three-way interaction among sleep quality, general work efficiency, and sensorimotor integration that we propose to study in astronauts and cosmonauts participating in the U.S./Russian Mir Program.

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 REM. 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, although 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 is the sequences of changes when subjects re-enter 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 re-adaptation to normal gravity.

Publications, Presentations, and Other Accomplishments:

Ajilore, O., Stickgold, R., Rittenhouse, C., and Hobson, J.A. Nightcap "Laboratory and home-based evaluation of a portable sleep monitor." Psychophysiology, 32, 92-98 (1995).

Datta, S. and Hobson, J.A. "Suppression of ponto-geniculo-occipital waves by neurotoxic lesions of pontine caudo-lateral peribrachial cells." Neuroscience, 67 (3), 703-712 (1995).

Stickgold, R. and Hobson, J.A. "Home monitoring of sleep onset and sleep onset mentation using the Nightcap. In: Sleep Onset: Normal and Abnormal Processes." Edited by: Ogilvie, R. and Harsh, J. APA Books: Washington DC, pp 141-160, 1995.

Stickgold, R., Neri, D.F., Pace-Schott, E., Juguilon, A., Czeisler, C.A., and Hobson, J.A. "Nightcap detection of decreased vigilance." Sleep Research, 24, 500 (1995).

Crew Member and Crew-Ground Interactions During NASA/Mir

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Funding:

Project Identification: E628
Solicitation: 94 OLMSA-01

Initial Funding Date: 9/95
Expiration: 9/98

FY 1995 Funding: $10,000
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

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 competed 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. There are no results to report for the FY95 period since funding for this study began during the last week of FY95 and the missions under study have not yet been launched.

Task progress for this period consists of initial start up activities such as grant administration and the initialization of the process for hiring personnel.

In planning for future manned space missions involving international crews of men and women, it it 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 1) astronaut and cosmonaut communication in space and 2) 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 stressed. 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.

Magnetic Resonance Imaging After Exposure to Microgravity

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Funding:

Project Identification: E586

Solicitation: 94 OLMSA-01

Initial Funding Date: 3/95

Expiration: 2/96

FY 1995 Funding: $6,666

Students Funded Under Research: 0

Flight Information:

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 8 days in weightlessness. Our published bed rest studies have documented the degree of expected atrophy after 4 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 bed rest studies have shown that when normal subjects are put in bed rest, partially unloading the spinal column, significant intervertebral disc expansion occurs. This expansion reverts to normal shortly after reambulation following bed rest lasting days to a few weeks. Longer duration bed rest (17 weeks) however, results in some residual expansion that remains for some time following reambulation. We have shown that 8 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 proposal 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.

Space flight measurements have documented that significant bone and muscle atrophy occurs during weightlessness. Knowledge of the extent and temporal relationships of the 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 one 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 often involves the intervertebral discs. Bed rest 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 bed rest on Earth, e.g., 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.

Analysis of Volatile Organic Compounds on Mir Station

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date: 10/95

Expiration: 9/98

FY 1995 Funding: $86,000

Students Funded Under Research: 4

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

The goal of this research is the characterization of volatile organic compounds (VOCs) in air samples from Mir Space Station using new technology based on ion trap mass spectrometry (ITMS). Twenty four hour time-averaged samples will be collected onto cartridges using the U.S. Solid Sorbent Air Samples (SSAS). Grab samples will be collected using U.S. Grab Sample Containers (GSC). Samples will be transferred from Mir via the Space Shuttle, forwarded to the Toxicology Laboratory at NASA Johnson Space Center (JSC) for analysis and sample subdivision, and then sent on to San Francisco State University (SFSU) for this 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.

Pete Palmer prepared and submitted an experiment document (ED) in early 1995. Palmer and John James (JSC) developed and signed a memorandum of understanding (MOU) which outlined the purpose of their collaboration and respective responsibilities. Palmer, Belisle, James, and Tom Limero (KRUG Life Sciences) completed 5 of the 6 supporting studies outlined in the ED. The JSC Toxicology group has already begun the collection and analysis of air samples from Mir. On October 27, the contract for this work was signed off by NASA. The signed contract was received at SFSU's Office of Research

and Sponsored Programs in November and an account was put into place in December. Contractual work on this project officially commenced in December.

The major accomplishment during this reporting period was the reassessment of the goals of this work as stated in the original proposal, ED, and MOU. Pete Palmer consulted with Warren Belisle on several occasions in December and January to define objectives, delineate equipment and supply needs, and delegate responsibilities of both investigators. Both Palmer and Belisle traveled to JSC in January to discuss these objectives with James and Limero and review the Toxicology Lab's standard procedures used for analysis of air samples from spacecraft environments.

Palmer and Belisle also began to put the requisite instrumentation for this work into place in their respective laboratories. Palmer also tested two different sample introduction systems for DSITMS. Preliminary results are very promising, with detection limits on the order of 50 parts-per-billion by volume, tailored selectivity through the use of selected ion monitoring and tandem mass spectrometry, and analysis times on the order of seconds.

Carla Remigi-Sanchez, a candidate for a master's degree in chemistry at SFSU, and Minhtram Nguyen, an undergraduate biochemistry major, are being brought up to speed on this project and trained in the use of GC/MS and DSITMS techniques for air analysis. Palmer gave a presentation on recent progress on DSITMS at the meeting of the Society of Western Analytical Professors. Remigi-Sanchez and Palmer submitted an abstract for a paper to be presented at a national conference on MS.

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 (ITMS). The research will provide 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. 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. Finally, the technology developed as part of this work will have potential use in a number of Earth-based applications involving air monitoring. These include atmospheric monitoring, ecosystems monitoring, stack monitoring, fence-post monitoring, hazardous waste site monitoring, and breath analysis.

The Effects of Long Duration Space Flight on Gaze Control

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir 1B

Responsible NASA Center: Johnson Space Center

Task Description:

Exposure to the stimulus rearranging conditions of space flight changes the efficacy of the eye-head coordination systems in their ability to localize and maintain fixation on static and dynamic visual targets. Such deficits compromise the capacity of humans to live and work with maximum effectiveness for short and especially for long periods of time in microgravity and increase the risk of hazard, both during on-orbit activities, as well as, during the entry, landing and egress phases of a mission. To understand these deficits, we propose six integrated sensorimotor experiments developed using, in part, tasks that have been under investigation as a part of the NASA Extended Duration Orbiter Medical Project (EDOMP), and identified as Detailed Supplemental Objective (DSO) 604, Operational Investigation 3 (OI-3). These experiments have been designed to investigate and characterize the evolution (or emergence) of goal-oriented strategies, and corresponding compensatory mechanisms, required to maintain effective gaze when the interactive sensorimotor systems necessary for gaze have been modified as a function of exposure to the stimulus rearrangement of space flight. We hypothesize in part: (1) that goal-oriented behavior in maintaining effective gaze will be modified by new strategies that maximize the positive aspects of visual dominance and the negative aspects of head movements during on-orbit performance, and immediate postflight behavior; (2) that control of the head's position in space will be compromised via modification in vestibular and proprioceptive

function, (3) that crew members' spatially oriented perception and consequent compensatory action initially exhibits increased reliance on extrinsic spacecraft coordinates (perhaps driven by the initial reliance on vision), but that an intrinsic coordinate system becomes more heavily weighted as mission duration increases; (4) that in space flight with gravity removed from the equation, orientation vectors may be established with reference to intrinsic and extrinsic coordinate systems that determine response vectors (i.e., the direction of the eye velocity vector during flight attempts to align with intrinsic coordinates, and that the primary axis of orientation, unlike that observed when the stimulus is aligned with gravity, is the body Z axis), and that once a head movement has been initiated, immediate control of the head's position in space will be compromised (due to space flight induced changes in vestibular and proprioception function), and that without appropriate feedback, target acquisition and other tasks requiring head control will be affected. It is our objective to use the following tasks, pre-/post- and inflight, to test the above hypotheses: (1) Target Acquisition, (2) Target Acquisition to remembered target positions, (3) Pursuit Tracking, (4) Sinusoidal Head Oscillations (head shakes), (5) Memorized Head Rotations, and (6) Test for both Spontaneous and Gaze Nystagmus. Results of this study will help in the development of countermeasures to alleviate the mal-effects of the described sensorimotor changes.

Equipment and software to accomplish all of the experiment functional objectives is now currently aboard the Mir Station. Baseline data collection hardware and flight training hardware have been installed at Star City.

Two sessions of baseline data collection have been completed with the Mir-23 prime crew. Both crew members are excellent subjects and understand the hardware and science requirements.

NASA's Mir Gaze experiment (E647), titled, "Effects of Long Duration Space Flight on Gaze Control", is a follow-on set of investigations developed from the Shuttle-Mir Science Project (SMSP) Phase 1A and EDOMP projects. The hardware required to support this experiment 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 modify completely 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 very long duration missions. This data is extremely valuable in assisting NASA advance to space station flights, and to assist in helping insure the safety, health and well being of future astronauts.

Publications, Presentations, and Other Accomplishments:

Huebner, W.P., Paloski, W.H., Reschke, M.F., and Bloomberg, J.J. "Geometric adjustments to account for eye eccentricity in processing horizontal and vertical eye and head movement data." Journal of Vestibular Research, 5(4), 299-322 (1995).

Reschke, M.F., Bloomberg, J.J., Harm, D.L., Paloski, W.H. "Space flight and neurovestibular adaptation." J. Clin. Pharmacol., 34, 609-617 (1994).

Reschke, M.F., Bloomberg, J.J., Paloski, W.H., Harm, D.L., Parker, D.E. "Neurophysiologic aspects: Sensory and sensory-motor function. In: Nicogossian, A.E., Leach, C.L., Pool, S.L., eds. Space Physiology and Medicine." Lea & Febiger, Philadelphia, PA, pp 261-285, 1994b.

Reschke, M.F., Harm, D.L., Bloomberg, J.J., and Paloski, W.H. "Chapter 7: Neurosensory and Sensory-Motor Function. In: A.M. Genin and C.L. Huntoon, eds. Space Biology and Medicine, Vol. 3: Humans in Spaceflight, Book 1: Effects of Microgravity." AIAA, Washington, DC, In press, 1995.

Reschke, M.F., Harm, D.L., Parker, D.E., Sandoz, G.R., Homick, J.L., Vanderploeg, J.M. "Neurophysiologic aspects: Space motion sickness. In: Nicogossian, A.E., Leach, C.L., Pool, S.L., eds. Space Physiology and Medicine." Lea & Febiger, Philadelphia, PA, pp 228-260, 1994a.

Assessment of Humoral Immune Function During Long Duration Space Flight

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Funding:

Project Identification: E621

Solicitation: 94 OLMSA-01

Initial Funding Date: 8/95

Expiration: 9/ 95

FY 1995 Funding: $28,000

Students Funded Under Research: 0

Flight Information:

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. The proposed experiments will improve our understanding of space flight-induced immune suppression.

First flight for this investigation will occur during FY 96 on STS 76/Mir 21. The investigation will utilize the same hardware developed for the Phase 1A Humoral Immunity experiment. Crew training activities and baseline data collection were performed late in FY95.

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.

Collecting Mir Source & Reclaimed Waters for Postflight Analysis

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Funding:

Project Identification: E592

Solicitation: 94 OLMSA-01

Initial Funding Date: 7/95

Expiration: 7/96

FY 1995 Funding: $237,178

Students Funded Under Research: 0

Flight Information:

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 crew members of the International Space Station (ITS) 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. To date, both U.S. and Russian programs have limited information on the composition of spacecraft and reclaimed water. 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 demonstrate U.S.-supplied hardware for collection of Mir water samples. Results of the analysis of water samples collected during the Mir 18 mission show that only up to 5% of the constituents of the reclaimed water could be identified using present analytical techniques. Some specific contaminants found were methylene chloride, chloroform, dioctyl phthalate, and formaldehyde. Results show the reclaimed water met all NASA water quality standards except for total organic carbon and methylene chloride.

Sampling Hardware Development
To allow source and reclaimed waters to be collected aboard Mir, sampling hardware that would mate U.S. hardware to the Mir SRV-K2 (galley) ports and the SVO-ZV (backup water supply system) ports had to be developed. In addition, sampling containers with the capacity to collect and store samples of water were also needed. For use on Mir 18, a 1-liter Teflon sample bag with a septum interface was developed. These bags were received with three sides sealed from a commercial vendor. A Teflon

septum adapter assembly and septum were sealed onto the fourth side of the bag at Johnson Space Center. These bags were then mated with a "potable water sampler." The potable water sampler consists of a teflon adapter that mates to the Mir SRV-K2 and SVO-ZV water ports and a stainless steel needle that mates with the U.S. water container. Unfortunately, these sample bags leaked during Mir 18 and, in some cases, not enough sample was available to perform a complete analysis. An investigation of the leakage problem revealed defective seals on the side and top seams of the bags.

New and improved sample bags have been developed for use during Mir 21 and subsequent missions. These new sample bags are commercially available, made of Teflon, and contain one polypropylene female luer lock port on each end of the bag. A polycarbonate reflux valve and a polyvinyl chloride cap are attached to the top luer lock port of the bag at Johnson Space Center. With the new interface on the sample bag, a new portable water sampler has also been developed. The samplers consist of a Teflon adapter that mates to the Hot/Cold or SVO-ZV dispenser port as appropriate, and a stainless steel male luer lock fitting that mates with the water bags.
Chemical analyses of 5 water samples collected during Mir 18 and STS-71 missions have been completed. These results include analysis for pH, conductivity, color, calcium, magnesium, silver, total organic carbon, alcohols, organic acids, semivolatiles, carboxylates, nonvolatiles, volatile organics, formaldehyde, amines, semivolatiles, and the preparation of organic carbon balances. Only 5 percent of the organic content in the water samples from Mir 18 could be identified using available analytical techniques, indicating the need for further development of analytical methods in order to more fully account for the total organic carbon.

Preliminary sample analyses on four potable water samples (three taken during Mir 19 and one taken during STS-74) and four humidity condensate samples (one taken during Mir 20 and three EDV samples from STS-74) have also been completed. Work yet to be completed for samples collected during Mir 19, STS-74 and Mir 20 include analyses for anions, cations, amines, and preparation of the organic carbon balances.

This research will provide benefits in the areas of methods development for the analysis of drinking water, advanced technologies for the treatment of waste waters, 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 tenfold over present techniques. These improvements will allow more complete characterization of potable water, accounting for nearly all organic constituents, even those at extremely low levels. In addition, by adapting techniques for treating spacecraft waters, the development of better waste water treatment technologies on Earth will be supported.

Publications, Presentations, and Other Accomplishments:

Homan, M.H., Mudgett, P.D., Schultz, J.R., and Sauer, R.L. "GC/MS and CE methods for the analysis of trace organic acids in reclaimed water supplies." Proceedings of the 24th International Conference on Environmental Systems, SAE #941392, Friedrichshafen, Germany, July 1994.

Straub II, J.E., Schultz, J.R., Michalek, W.F., and Sauer, R.L. "Further characterization and multifiltration treatment of Shuttle humidity condensate." Proceedings of the 25th International Conference on Environmental Systems, SAE #951685, San Diego, CA, July 1995.

Bone Mineral Loss and Recovery after Shuttle/MIR Flights

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Funding:

Project Identification:

Solicitation: 94-OLMSA-01

Initial Funding Date: 4/95

Expiration: 9/00

FY 1995 Funding: $80,000

Students Funded Under Research: 0

Flight Information:

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, however, only limited data on the very important issue of recovery of bone after return to one-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.

Work conducted in support of E598 has included the writing of supporting documentation, crew training/familiarization, intercalibration checks of experiment hardware, continued quality control checks of experiment hardware, and Baseline Data Collection for Mir 21 prime and backup crew members. Milestones set for this experiment have been met on schedule. Future work on this task is expected to proceed on schedule as well.

Progress in FY95 included writing (and revising as necessary) the following documents: Experiment Document, Crew Familiarization presentation materials, Crew Training Protocol, Integrated Payload Requirements Document, Baseline Data Collection Requirements Document, Experiment Manual, JSC IRB Master Protocol and Informed Consent forms, and a detailed multi-year budget. Prime and backup crew members attended an experiment briefing, which provided an overview of the study purpose and test procedures. The whole body densitometers in the U.S. and those on-loan to Russia were tested to assess the degree of intercalibration. Dosimetry checks on all of the densitometers were conducted as well. Regular scanning of spine, hip, and whole body phantoms was conducted (and still continues) on all densitometers to assure stability of instrument performance. The Russian prime and backup crew members participated in the first of two Baseline Data Collections for bone densitometry (DEXA), muscle strength testing (LIDO), and blood and urine testing for markers of bone metabolism (e.g., markers of bone formation and bone resorption). DEXA scan regions included the whole body, lumbar spine, hip, and calcaneus. LIDO isokinetic strength testing was performed on the back, knee, and ankle.

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.

Evaluation of Skeletal Muscle Performance and Characteristics

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date: 10/94

Expiration: 9/98

FY 1995 Funding: $40,000

Students Funded Under Research: 0

Flight Information:

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. Reduced fitness causes decreases in strength, endurance, tone, and efficiency. 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.

- Experiment documentation manual was completed.
- Submitted protocol and procedures to the JSC IRB.
- Completed the development of the training and procedure manual for inflight experiments, currently under review by Russian co-investigators and trainers.
- Completed development of the Experiment manual.
- Began training of Mir-22/NASA-3 crew members.

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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Funding:

Project Identification: E613

Solicitation: 94 OLMSA-01

Initial Funding Date: 6/95

Expiration: 12/95

FY 1995 Funding: $25,400

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

The primary objective of this project is to determine the duration of the metabolic stress response associated with space flight. The secondary objective is to determine how long it takes for protein metabolism to return to its preflight state after a long duration mission. We plan to accomplish these goals by measuring the whole body protein synthesis rate, three times before, four times during space flight (duration 90-180 d) and four times after space flight up until 45 days after landing. 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 postflight measurements are to determine how long it takes for the whole body protein synthesis rate to return to the preflight baseline.

During FY 1995 the following tasks were accomplished: a detailed protocol was agreed upon with the Russians; the NASA 2/Mir 21 crew were briefed on the experiment; one of the three baseline data points (L-120) was collected on the two MIR 21 crew persons and the NASA 2 subject at the end of September in Moscow. Data collection went well and no problems were encountered.

During FY 1996 we expect to complete the baseline, flight and postflight data collection on these three subjects. The fact that the first data collection sessions proceeded without any problems being encountered augurs well for future sessions.

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 preprogrammed specific response?

Renal Stone Risk During Long Duration Space Flight

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Funding:

Project Identification:

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1998

FY 1995 Funding: $80,000

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: Johnson Space Center

Task Description:

This investigation is a continuation of the study begun with the Mir 18 mission (Shuttle Mir, Phase 1A). Data from previous missions suggests that space flight exposes crew members to a greater risk of forming kidney stones. Investigators believe that the risk increases with the duration of the mission. The investigation attempts to determine the degree of risk involved during extended space flight and to determine the factors which are affected by flight duration. Ultimately, medical investigators hope to use their understanding of increased in-flight kidney stone risk to determine ways to counteract the formation of these stones both in space and on Earth.

This investigation is currently manifested for the Mir 21 (launch March 1996) and Mir 23 long duration space missions. Flight hardware to support this study was delivered by the crew of STS-74, and is aboard the Mir Space Station awaiting arrival of the Mir 21 crew. Two preflight baseline data collections have been completed with a third scheduled for February 1996. Data analyses is continuing.

Approximately 12 percent of the Earth-bound population will develop a renal stone sometime during their lives. Initially, lessons learned from studies on Earth will be used to minimize the potential for renal stone formation in crew members 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 crew members.

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. 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. As an example, currently under development is a method of urine collection in which the urine is dried on a filter card, uses no preservatives, and can be stored at ambient temperatures for extended periods of time.

Neuro-Thyroid Ineteraction on Skeletal Isomyosin Expression in 0 g

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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 9/95

Expiration:

FY 1995 Funding: $110,738

Students Funded Under Research: 3

Joint Participation: NIH/National Institute of Neurological Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Ames Research Center

Task Description:

The Goal of this project is to examine the interactive role of gravity, enervation, and thyroid hormone (T#) 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 MHC isoforms. 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.

During FY 95 the primary tasks undertaken on this proposal have been 1) to define the conditions implanting pumps into nursing Dams in order to induce a hypothyroid state that is transferable to the suckling pups; 2) to develop extraction procedures for isolating total RNA, DNA, and protein for biochemical and molecular analyses on small quantities of tissue; and 3) to initiate assays for myosin heavy chain (MHC) mRNA analysis using the technique of reverse transcriptase polymerization chain reaction (RTPCR) on small tissues samples.

Our accomplishments are as follows. First, we have partially succeeded in inducing a hypothyroid state in rodent pups by infusing methimazole via osmotic pumps into the Dams (50 mg/kg/day). These neonatal animal have smaller heart m, and a different cardiac MHC profile from control (euthyroid) animals indicating that we are achieving a hypothyroid state. However, we are concerned that the pups are consuming food crumbs that the mother breaks off during feeding that may compromise their complete dependence on the mother's milk for nourishment. Consequently, we are in the process of doing a follow-up project in which the mother's diet is provided by the food bars actually used for space flight. These food bars have a gummy texture so that little or no food is broken off and are thus available to the pups. This should eliminate food spillage and insure that the animals are nourished via the milk thereby optimizing methimazole transfer to the pup.

Second, we have succeeded in developing an extraction procedure to isolate total RNA, DNA, and protein (denatured) for analyses of MHC mRNA analysis, MHC protein analysis via gel electrophoresis, and DNA for normalizing the data. This will be the primary method for isolating these constituents in the space flight project.

Third, we are in the process of setting up the RTPCR techniques to quantify the mRNA signal for the neonatal MHC and for the 4 adult skeletal muscle MHCs. We have identified the appropriate primers for each MHC and have showed that the RTPCR reaction is specific to the types of muscle we will be studying. Our goal now is to develop internal standards so that we can quantify the mRNA from one sample to another.

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 myosin heavy chain (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.

Thus, these experiments will for the first time delineate how gravity impacts an important developmental and maturation process affecting 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.

Publications, Presentations, and Other Accomplishments:

Baldwin, K.M. "Future research directions in seeking countermeasures to weightlessness." J. Gravitational Physiol, 2, 51-53, 1995.

Baldwin, K.M. "Effect of spaceflight on the functiional, biochemical, and metabolic properties of skeletal muscle." Med. Sci. Sport and Exercise, 1995.

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Integration of Neural Cardiovascular Control in Space

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Funding:

Project Identification: E294 & E023

Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94

Expiration: 9/99

FY 1995 Funding: $98,248

Students Funded Under Research: 0

Joint Participation: NIH/National Institute of Neurological Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Johnson Space Center

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 lG. 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.

Four separate proposed experiments have been integrated into a joint experiment on cardiovascular autonomic control to be carried out on Neurolab in 1998. The joint experiment includes Baisch et al. (DLR, Cologne, Germany): Artificial Neural Networks and Cardiovascular Regulation, Eckberg et al. (Medical College of Virginia, Richmond, VA): Autonomic Neuroplasticity in Weightlessness, and Robertson et al. (Vanderbilt University, Nashville, TN): Autonomic Mechanisms in Microgravity.

Detailed combined protocols have been developed and approved by the human use committee at JSC. Instrumentation has been defined. Detailed plans for supporting ground-based studies and for crew training have been defined.

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.

Publications, Presentations, and Other Accomplishments:

Arbeille, Ph., Gaffney, F.A., Beck, L., Coulon, J., Porcher, M., and Blomqvist, C.G. "Effect of microgravity on renal and femoral hemodynamics during lower body negative pressure and intravenous saline load." Proceedings of the Norderney Symposium on Scientific Results of the German Spacelab Mission D-2. P.R. Sahm, M.H. Keller, B. Schiewe (eds.). Wissenschaftliche Projektführung D-2: Köln, Germany, pp. 679-681, 1995.

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Blomqvist, C.G., Levine, B.D., Lane, L.D., and Buckey, J.C. "Space medicine and physiology. In: Current Medicine. Atlas of Heart Disease Series." Edited by: Braunwald, E. Mosby, Inc.: Philadelphia, PA, In Press, 1995.

Buckey, J.C., Gaffney, F.A., Lane, L.D., Levine, B.D., Watenpaugh, D.E., Wright, S.J., Yancy, Jr., C.W., Meyer, D., and Blomqvist, C.G. "Central venous pressure in space." J. Appl. Physiol., (In Press).

Buckey, J.C., Lane, L.D., Levine, B.D., Watenpaugh, D.E., Wright, S.J., Moore, W.E., Gaffney, F.A., and Blomqvist, C.G. " Orthostatic intolerance after spaceflight." J. Appl. Physiol., (In Press).

Levine, B.D., Lane, L.D., Gaffney, F.A., Buckey, J.C., and Blomqvist, C.G. "Maximal exercise performance after adaptation to microgravity (Abstract)." Med Sci Sports Exercise 26:S112, 1994.

Levine, B.D., Lane, L.D., Watenpaugh, D.E., Gaffney, F.A., Buckey, J.C., and Blomqvist, C.G. "Maximal exercise performance after adaptation to microgravity." J. Appl. Physiol., (In Press).

Space Flight, Stress, and Neuronal Plasticity

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Funding:

Project Identification:

Solicitation: AO 93-OLMSA-01

Initial Funding Date: 8/95

Expiration:

FY 1995 Funding: $80,311

Students Funded Under Research: 1

Joint Participation: NIH/National Institute on Aging

Flight Information:

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 to the conditions of space flight.

Funding for experimental work on this project only began on 1 September 1995. The major component and the primary flight component involves a study of stress on functional neuronal

architecture in mice. Since this component was a new initiative, substantial groundwork is needed before these studies are fully underway. At present, we have been establishing the ground-based studies and are beginning to refine protocols for eventual application to space flight animals. The necessary reagents and equipment are being obtained or developed. Some equipment (microplate reader) has been ordered, but has not yet arrived. Baseline studies are now being done on control animals. We have established the validity of ELISAs for quantitative analyses of cytoskeletal proteins in different brain regions. Preliminary experiments on the administration of corticosteroids and drugs are underway. One person is currently being trained to work on this project and additional personnel are being actively recruited. Previous work on presynaptic function in the squid giant synapse is still underway. Although no additional data will be gathered until the summer of 1996, data gathered from previous summers is currently being analyzed and prepared for publication.

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 an 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, first to understand the molecular basis of neurological changes associated with stress and, second 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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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Joint Participation: National Science Foundation

Flight Information:

Flight Assignment: Small Payload (TBD)

Responsible NASA Center: Ames Research Center

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 documenting 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.

Studies during the early phases of this project were designed to address four feasibility issues: 1) Can Zebrafish embryos survive in a closed system such as the ECU for the duration of the flight? 2) How

early in development can the embryos reach microgravity, given that the eggs must be fertilized on the ground and given the timing constraints of the shuttle launch? 3) Can the fixed embryos remain at 22-24 degrees C for the remainder of the flight without degradation of their morphology? 4) Can the embryos survive the shuttle launch and continue normal development?

Embryo survival in the Egg Chamber Units: For all egg chamber tests, 50 eggs with chorions enzymatically removed at 80% epiboly were placed in ECUs containing 10% Hanks with 30 μg/ml phenylthiourea added to prevent melanin formation. 2 mls paramecium culture was added as a food source and to prevent bacterial proliferation. The chambers were sealed and placed at 22-24 degrees C, and embryo survival was assessed after 2 weeks, the proposed duration of the flight. In 10 tests, embryo survival rates varied from 20-50% as compared to 50-75% survival for embryos raised under normal laboratory conditions. Thus in a worst case scenario aboard the shuttle there would be 10 specimens collected at each time point which should be sufficient to fulfill the science requirements.

Developmental timing studies: In order to ensure that the Zebrafish will be able to reach microgravity before the primary vestibular afferents begin to grow into the hindbrain, various cooling paradigms were tested to slow down the embryos development. Zebrafish raised at 18 degrees C showed normal survival and developed normally but slowly. The rate of development of the embryos at this cold temperature is about 0.4 times the normal developmental rate. Therefore if a shuttle launch delay results in the maximum waiting period between loading the embryos on board the shuttle and reaching microgravity, a delay of 48 hours, the fish would still be at a developmental age of -27 hours. At this age the hair cells of the otolithic maculae are just beginning to develop and primary afferent fibers have not yet begun their growth into the hindbrain.

Fixation studies: In order to determine whether the fixed embryos on board the shuttle could remain in the same Refrigeration/Incubation Module with the still living specimens, or whether refrigeration of the fixed-embryos would be necessary, Zebrafish embryos at 1, 3, 7 and 10 days of development were fixed in 4% paraformaldehyde and left at 22-24 degrees C for 2 weeks. The fixed specimens were then observed at 20 X to check for degradation of gross morphology, and then primary afferent fibers were labeled. Morphology of all the specimens appeared normal, and Dil transport and afferent arbor patterns were unaffected.

Shuttle launch simulation studies: In order to determine whether Zebrafish embryos would be adversely affected by the shuttle launch, launch simulation tests were carried out on the 20G centrifuge at NASA's Ames Research Center. In these tests live Zebrafish embryos were subjected to the gravitational profile and acoustic noise levels which occur during an actual shuttle launch. Two age groups were used, 20 hours and 28 hours. These ages were chosen because they represent the earliest and the latest stages at which the embryos would experience the launch, depending on whether there is a launch slip or not. Two hundred embryos were used in each test group. All embryos survived the launch simulation. Gross morphology was normal, as was semi-circular canal wall fusion and otolith development. The inner ears of 10 fish from each group were injected with the specific hair cell label 4-Di-2-ASP in order to assess the development of the vestibular end organs. In all cases the end-organ development appeared normal. At one week embryos were sacrificed and fixed in 4% paraformaldehyde, and individual vestibular end-organs in several embryos from each experimental group were injected with Dil to label the primary vestibular afferents. In all cases afferent projection patterns and axonal arborizations appeared normal.

It thus seems that Zebrafish embryos involved in the proposed flight experiments will not be adversely affected by the shuttle launch. Our investigation of the influence of microgravity on the development of specific neuronal connections in the vestibular system both will answer fundamental questions about the effects of changes in the sensory environment on the establishment of partway specificity in the nervous system.

Normal development of the vestibular system at 1G 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 it 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 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.

Publications, Presentations, and Other Accomplishments:

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Chapman, B. and Fraser, S.E. "Vestibular hair cells develop normally in zebrafish embryos where canal wall fusion has been disrupted." Meeting on Zebrafish Development and Genetics, Cold Spring Harbor, April 1994.

Fraser, S.F. and Chapman, B. "Axonal arborizations of the primary afferents serving different vestibular end-organs in zebrafish embryos." Soc. Neurosci. Abstr., 20, 1684 (1994).

Spatial Orientation of Vestibulo-Occular Reflex and Velocity Storage

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Funding:

Project Identification: E047

Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94

Expiration: 12/99

FY 1995 Funding: $119,830

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 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. Experiments are proposed to determine how microgravity affects the orientation vectors of OKN and the VOR of human subjects, and how the vectors are altered by introduction of linear forces due to centrifugal acceleration. There was also long-term depression of compensatory ocular counter-rolling (OCR) after space flight in monkeys on the COSMOS 2229 Flight. We postulate that there will be depression of compensatory ocular torsion movements after adaptation to space, and propose to study torsion eye movements before, during and after flight. Eye movements will be recorded during horizontal, vertical and oblique OKN and during rotation with the head upright and tilted. OKN will be induced with a binocular optokinetic stimulator and linear forces will be induced by centrifugation in the MVI rotator. The axes of eye rotation will be calculated using a model-based approach. Subjective and objective measures will be used to measure static ocular tilt on earth, and off-vertical axis rotation (OVAR) will be utilized in ground-based testing to produce dynamic OCR, vertical eye movements and post-rotatory nystagmus from tilted positions.

A new video technique will be utilized in ground-based testing to study OCR. 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.

Planning of the experiments is completed. We now have started to do the Ground Based Studies that will form the basis for the Neurolab flight.

Centrifuge: We will shortly let a contract for a short-arm centrifuge that will be used in Ground Based Testing. The rotator has been designed, but there are some issues to be resolved. The contractor will travel to Bordeaux to meet with Aerospatiale to accomplish this. We expect delivery on the rotator in September, 1996. This rotator will be used for the Ground-Based Studies at Mount Sinai Medical Center in New York. It will then be shipped to JSC at Houston where it will be utilized for the Baseline Data Collection and for the Postflight Data Collection.

Eye Movement Recording System: A laboratory version of the eye recording measurement system (EMRS) is being built by Drs. Theodore Raphan and Steven Moore to support the Ground-Based Studies at Mount Sinai, and the pre- and postflight data collection at the Johnson Space Center.

Off-Vertical Axis Rotation: A tilting, rotating chair that will be used to deliver off-vertical axis rotation is available at the JFK Medical Center (Dr. Martin Gizzi, Co-Investigator). This will be used for the Ground Based Testing with a three-dimensional eye movement recording device. An OVAR chair at JSC has also been identified that will be used in the Baseline and Postflight Data Collection.

The equipment being built by ESA (Centrifuge, Eye Movement Recording System, Eye Stimulation System) is generally on or close to schedule. We should receive a working copy of the flight eye movement recording system and eye stimulation system in November, 1996. ESA and the PI's will monitor whether the binocular three-dimensional eye movement recording system will work as specified. Since this is new technology and the time for testing before flight will be very short, this is an issue of concern.

It should be noted that this is a true international project in every sense of the word. The team of investigators involves the USA (New York and Brooklyn), France, Australia and Japan. We are in constant contact by email. Dr. Curthoys of Australia made a major contribution to the project by sending a Postdoctoral Fellow, Steven Moore, who did his thesis research on video-based, three-dimensional eye movement recordings. Dr. Moore is already working on this project, and will provide very important help in achieving our goals.

The proposed research will determine how otolith-ocular reflexes and spatial orientation of the angular vestibulo-ocular reflex (aVOR) 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 1g 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 ocular counter-rolling (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 countermethods 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.

Studies on vergence will be done in the present experiments. If there were problems with verging the eyes while moving toward targets after landing, it could have important function significance.

A major advance will be development of a three-dimensional model of the VOR which will include both angular and linear acceleration inputs, and will account for dynamic changes that alter the orientation of the system vectors to the vector 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 all conditions in which there is postural imbalance or gaze instability. An example where such information will have important clinical significance is in postural imbalance of the elderly.

We are developing a new three-dimensional, binocular video technique for recording eye movements that has 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.

Publications, Presentations, and Other Accomplishments:

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Dai, M., McGarvie, L., Kozlovskaya, I.B., Raphan, T., and Cohen, B. "Effects of spaceflight on ocular counterrolling and spatial orientation of the vestibular system." Exp. Brain Res., 102, 45-56 (1994).

Gizzi, M., Raphan, T., Rudolph, S. and Cohen, B. "Orientation of human optokinetic nystagmus to gravity: a model-based approach." Exp. Brain Res., 99, 347-360 (1994).

Clinical Trial of Melatonin as Hypnotic for Neurolab Crew

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Funding:

Project Identification: E104

Solicitation: 93 OLMSA-01

Initial Funding Date: 10/94

Expiration: 2/99

FY 1995 Funding: $62,363

Students Funded Under Research: 2

Joint Participation: NIH/National Institute on Aging

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Johnson Space Center

Task Description:

Sleep disruption is common during space flight. A survey of 58 crew members from 9 shuttle missions revealed that most suffered from sleep disruption and were unable to sleep more than six hours per day of flight as compared to 7.9 hours per day on the ground. Nineteen percent of crew members on single shift missions and 50 percent of the crew members in dual shift operations reported sleeping pill usage (benzodiazepines) during their missions. Although benzodiazepines are effective as hypnotics, their adverse next-day side effects include sedation, performance decrements, amnesia and distortions in the sleep EEG.

Our preliminary data suggest that the pineal hormone melatonin, which has been reported to modulate the output of the human circadian pacemaker, may also have the acute hypnotic properties needed for treating the sleep disruption of space flight, without producing the adverse side effects associated with benzodiazepines. We hypothesize that pre-sleep administration of melatonin will result in decreased sleep latency, reduced nocturnal sleep disruption, improved sleep efficiency and enhanced next-day alertness and cognitive performance both in ground-based simulations and during the Neurolab mission.

Double-blind placebo-controlled trials are proposed in which: (1) the effectiveness of melatonin as a hypnotic is assessed independent of its effects on the phase of the endogenous circadian pacemaker in

ground-based studies, using a powerful experimental model of the dyssomnia of space flight; and (2) the effectiveness of melatonin as a hypnotic is assessed during the Neurolab mission. In both experiments the effects of melatonin on sleep stages and spectral composition of the EEG during sleep will be determined as well as its effects on daytime alertness and performance.

During FY95 we completed the experiment definition for the inflight protocol to examine the hypnotic effects of melatonin on Neurolab crew. Through continual collaboration with the Experiment Support Scientist (ESS), Payload Project Manager, and other Sleep Team members at UCSD, NASA and Lockheed Martin we documented and revised the experiment requirements, redesigning the protocol to meet all inflight time restrictions. By the end of the year we finalized changes to the inflight protocol and produced Training and Master Protocols. We also completed negotiations on the contract and budget for the development phase of the project.

Specifically, at the second Investigator Working Group (IWG) meeting in February we developed our selection criteria for payload specialists and later forwarded our nomination for payload crew member to NASA. As a result of our participation in the Mock-Up Demonstration later in the same month we made several changes to our inflight procedures to facilitate their integration including hypotheses, experimental design, data collection procedures, and required hardware at the Experiment Requirements Review (ERR) in March. Based on the results of that review, we revised the relevant sections of the Experiment Document (ED), submitting the changes to NASA for baselining. At the third IWG in August we participated in the selection of payload crew candidates. During the subsequent payload candidate screening process we coordinated the administration of polysomnographic examinations at various locations around the world, collecting and presenting the data at the fourth IWG. We presented our current experiment configuration at the Preliminary Design Review (PDR) in August, concentrating on hardware issues. Following that meeting we baselined additional sections in the ED for incorporation into the larger Integrated Experiment Document (IED). Late in the fiscal year we completed the Training Protocol describing the crew preflight orientation and training procedures. At the same time we drafted the Master Protocol for submission to NASA's Human Subjects Committee. By the end of the fiscal year we had finalized all the experimental details and budgets for both the hardware validation (ground-based) and inflight studies. Throughout the fiscal year we continued to work closely with Physiometrix, Inc. in the modification of the E-net, a newly designed electrode cap, for use by the payload crew during sleep periods in space.

In summary, at the end of this cycle the inflight experiment has been fully defined, all relevant sections of the ED have been baselined. Master and Training Protocols have been written, a protocol for the ground based studies to validate the modified e-net has been finalized, and contract negotiations for the development phase have been completed.

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:

Project Identification: E049
Solicitation: 93 OLMSA-01

Initial Funding Date: 9/95
Expiration:

FY 1995 Funding: $82,297
Students Funded Under Research: 3

Joint Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 that 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, much progress has been made regarding the organizational aspects of this complicated project. Experimental integration with other members of the Autonomic Control Team is complete. Inflight hardware has been defined, and steps have been taken to test the equipment astronauts will be asked to use during flight. One of the team's primary concerns (the measurement of muscle sympathetic nerve traffic during lower body negative pressure in space) is close to being resolved. Ground-based studies designed to address specific scientific aspects of each experimental protocol are currently underway.

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 attract 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.

Publications, Presentations, and Other Accomplishments:

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Smith, M.L., Beightol, L.A., Fritsch-Yelle, J.M., Ellenbogen, K.A., Porter, T.R., and Eckberg, D.L. "Valsalva's maneuver revisited -- insights into central modulation of human autonomic cardiovascular outflow." Am. J. Physiol., (In Press).

Smith, M.L., Fritsch, J.M., and Eckberg, D.L. "Rapid adaptation of vagal baroreflexes in humans." J. Autonom. Nerv. Syst., 47, 75-82 (1994).

CNS Control of Rhythms and Homeostasis during Spaceflight

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Funding:

Project Identification: Solicitation: 93 OLMSA-01

Initial Funding Date: 10/95 Expiration:

FY 1995 Funding: $25,000 Students Funded Under Research: 6

Joint Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 proposal 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. (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 the first year of the NASA Grant, Effect of Gravity on the Regulation of Circadian Rhythms, several of the specific aims have been accomplished. Our previous studies have examined the circadian rhythms and demonstrated that a one hour phase shifting light pulse will induce significant c-Fos expression within SCN neurons in Sprague-Dawley and Wistar rats. However, the rodent model chosen for Neurolab was the Fisher 344 strain of which this laboratory has not examined. Therefore, the first series of studies examined the suitability of the Fisher 344 rat for the purposes of our proposed flight experiments. Fisher 344 rats were implanted with biotelemetry units to measure body temperature and activity. The circadian rhythms of the Fisher 344 rats did not exhibit as consistent circadian rhythms periods or stability of entrainment as other strains. Additional analysis will be necessary to determine how well circadian measures can be quantified in this rat strain. We have also examined the effect of a one hour light pulse on c-Fos expression in SCN neurons of Fisher 344 rats. Fisher 344 rats exhibited the normal increase in c-Fos expression following a one hour phase shifting light pulse, but may not be as intense as other strains. There have also been several studies that have examined the protocols that will be used for Neurolab. We have tested the adequacy of using immersion fixation of the brain in 4% paraformaldehyde for immunohistochemistry histology. Although the background of the stained tissue was slightly greater, the procedure appears adequate for our needs. We have also been testing the surgical procedures and flight hardware that will record the circadian rhythms of the flight and control rats. We have implanted a number of animals and trained personnel on the surgical protocol. Several improvements in our surgical procedures have increased efficiency, reduced surgical time, and improved recovery time of the rats from surgery. The rats that have been recorded show that the transmitter is working. However, the recording hardware has not been adequate for reliable measures of rhythms.

The results from the research to date complement the other planned supporting studies for FY96. We will examine c-Fos expression in the SCN of rats that have been exposed to a light pulse of different intensities and length to determine the optimal flight protocol. In addition, we will continue to test the flight hardware so that it will be adequate for rhythm analysis. It will be necessary to continue our examination of the Fisher 344 rats for circadian rhythms to be sure that they are consistent and stable during entrainment and constant lighting conditions. We will also modify 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:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 12/95

Expiration:

FY 1995 Funding: $99,150

Students Funded Under Research: 2

Joint Participation: National Science Foundation

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Ames Research Center

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 and 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 to date has resulted in the refinement of the implantable wafer electrode and its cable and interconnect. We have implanted 10 animals and succeeded in recording neural activity from three. Our best case lasted six months but recently pulled its implant away from its anchor on the skull. We presently have five new animals implanted and are awaiting testing of their responses. If one of these animals demonstrates neural activity we will transport it to Japan for participation in the parabolic flight experiments in early March.

We continue to study the time course and biology of regeneration of severed VIIIth nerves. This is highly important because it is regenerated fibers that grow through our wafer electrode to provide the biological neural signals for study. It is important to establish whether regenerated nerves have the same biological, neurophysiological, and electrophysiological properties as normal, unsevered nerves to establish the validity of our model system. Studies do indicate that regenerated fibers appear completely normal. Several manuscripts are in preparation.

We are continuing to experiment with perfecting the wafer electrode. The present design is in a "spade" configuration and is inserted into a small cut transverse to the nerve. The University of Michigan is also manufacturing a round electrode that will be fitted with tubes to guide nerve growth through the electrode. The material composing these tubes is presently under study at the University of Utah and we hope to have electrodes of this design to implant later this year.

A major stumbling block has been the design of the electronics to transmit the nerve signals and acceleration signals from the animal to a recording device. Because the experimental protocol requires continuous recording before, during, and following the flight a battery powered amplification and transmission system is impractical. Therefore, in collaboration with Max Deffenbaugh, an MIT graduate student, we have developed a system for inducing power into the amplification and transmission system. Hopefully our Japanese partners are perfecting this technology and we await the Critical Design Review at a later date this year.

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 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.

Publications, Presentations, and Other Accomplishments:

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Anatomical Studies of Central Vestibular Adaptation

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Funding:

Project Identification: Solicitation: 93 OLMSA-01

Initial Funding Date: Expiration:

FY 1995 Funding: $ Students Funded Under Research: 0

Joint Participation: NIH/National Institute on Deafness and other Communication Disorders

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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.

No additional data was provided by the investigator for this research.

Effects of Space Flight on Drosophila Neural Development

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94

Expiration:

FY 1995 Funding: $72,162

Students Funded Under Research: 3

Joint Participation: National Science Foundation

Flight Information:

Flight Assignment: Small Payload (TBD)

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 Al 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 our 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.

The project being performed for Neurolab has several important ground-based development goals, for which we are making important progress. First, we wish to define the appropriate developmental stages for study by calibrating the temperature shifts we will do to expose the embryos and larvae to microgravity at specific times during development. This has been placed on hold by us due to the fact that the project will be flown as a small payload on a shuttle mission still to be determined. As we do not have information about the expected flight, we are not at present (1/96) able to make the precise determinations for the times when temperature shifts should be made. This is because we currently have no information about mission duration, which flight days and times the crew will be available to perform the experiment, or which temperature incubators will be available for our project. Once this information is available, we will be able to proceed promptly with this aspect of the study.

The second goal for this project is to develop *Drosophila* lines suitable for mass analysis of neuromuscular development. In this direction we have made extensive progress during 1995. In particular, we are examining lines using both heterologous promoters such as the GAL4-UAS system, as well as transgenes expressing specific reporter constructs with expression in subsets of cells in the developing system. As a pilot for this, we have made a P-element transformation vector using the green fluorescent protein (GFP) as a reporter (see below), which will allow scoring of neural anatomy en masse in whole mount using fluorescent microscopy without the need for either dissection or specific labeling. In addition, we have begun examining GAL4 lines using the mutant GFP protein, which is a significantly brighter reporter. 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 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 Green Fluorescent Protein (GFP) probes of the jellyfish *Aequorea victoria* . GFP is intensely fluorescent and shows relatively little photoinactivation. We have constructed a P-element transformation vector bearing a lacZ-GFP fusion gene under the control of a minimal hsp70 promoter. A polylinker upstream of the promoter allows for the insertion of enhancer sequences to drive expression of the gene. Our initial pilot work has been to create two lines, using either the mesoderm-specific or ectodermal regions of the Toll enhancer. We chose the Toll enhancer for reasons related to other studies in the lab, but it has proven to be a good pilot for other enhancers we will use for the Neurolab mission. The mesodermal enhancer drives expression of a lacZ reporter gene in a subset of ventral muscle fibers and their precursors beginning at approximately stage 12. The CPLG reporter we have constructed will be an effective marker for vital studies, using GFP as the reporter. It should be noted that it is possible to generate a variety of different expression patterns by selecting appropriate enhancer sequences. A wide array of suitable enhancers are available for *Drosophila*, and we are planning to use them to generate lines for the Neurolab mission. The status of this project at the time of writing is that two transformation vectors bearing the mesoderm-specific and the epidermal-specific Toll enhancer elements have already been prepared and we are doing embryo injections, with a goal to have stable germline transformants available for preliminary tests by winter 1996. Another 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 will

use a *Drosophila* line where the regulatory UAS sequences have been fused to the coding region of GFP. We are currently using two UAS-GFP lines. The first is a kind gift of G. Boulianne, University of Toronto, Toronto, Canada, and the second contains the mutant form of GFP, developed by Barry Dickson, U. Calif., Berkeley. A wide array of GAL4 lines suitable for labeling the developing CNS are available, and we are also examining lines with ubiquitous expression in the CNS and periphery, such as the GAL4-elav line. 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.

We have obtained our color CCD camera, and it has been integrated into our video imaging system. This new hardware is making it possible to easily archive anatomy from double-labeling experiments. We plan in the coming year to upgrade our image capture hardware to 24bit color, and to add to our morphometric software.

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 International 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.

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.

Publications, Presentations, and Other Accomplishments:

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Halfon, M., C. Hashimoto, H. Keshishian "The *Drosophila* Toll gene functions zygotically and is necessary for proper motoneuron and muscle development." Devel. Biol., 169, 151-167 (1995).

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Neuronal Development Under Conditions of Space Flight

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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 7/94

Expiration:

FY 1995 Funding: $39,662

Students Funded Under Research: 1

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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. If a child does not use one eye early in life because it is not aligned with the other eye, a very common condition called strabismus may cause blindness in the unused eye. Even after the eyes are realigned vision may not be restored. If visual input does not occur during the critical period, then sight may be lost for life. Our question in this study is whether the sensory information provided by gravity after birth is necessary for the development of spatial ability. We will take a first step toward answering this question by first studying the structure and function of certain brain areas. We will determine whether animals returning from space have a normal number of synapses, the connections between brain cells; whether certain key molecules become mature as in earth-based animals; and whether they develop the connections necessary to express memory circuits. These studies will tell us whether the brain has developed normally.

We have developed a tentative dissection procedure to secure tissue from the regions of the brain that may be affected by space flight. Tissue samples from key regions were placed in a special flask to retain a cold temperature for 3 days. To determine whether we could assess the maturation of certain key molecules, we extracted RNA from these tissue fragments, followed by a technique called PCR to determine whether the RNA for the specific molecules of interest were present and intact. We found the technique was successful for detecting the molecules of interest. Tissue was fixed under various conditions to determine whether we will be able to observe and count synapses. While the tissue was adequate for these purposes, certain morphological features were obscured by the fixation conditions.

Ensemble Neural Coding of Place and Direction in Zero-G

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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94

Expiration:

FY 1995 Funding: $102,658

Students Funded Under Research: 3

Joint Participation: NIH and Office of Naval Research

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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). We realistically expect to be able to obtain well isolated unit recordings from as many as 1000 neocortical, thalamic and tectal neurons over the course of a single mission, and to study the ensemble interactions of 50-150 cells in any given recording experiment.

The feasibility of performing behavioral tasks in 0-g were verified in KC-135 tests. Refinements of the implant hardware are near completion. Testing of the Research Animal Holding Facility (RAHF) cage suitability for rats with implants is underway. Refinement of behavioral task hardware is

underway. Development of a electronic and computer system for data acquisition is proceeding slightly behind schedule due to funding disruption. It is anticipated that a working prototype will be available for a KC-135 flight test in late Spring 1996.

The research seeks answers to fundamental questions about the brain mechanisms for the development of high level cognition maps of the world. The same neural structures are also involved in the establishment of long-term 'episodic' memories of experience. The knowledge obtained will aid in the development of better conceptual models for the neural basis of these phenomena and hence in the development, ultimately, of ameliorative treatments for deficits in these processes resulting from developmental disorders, brain trauma, drug abuse, disease and normal aging.

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Edwards, F.A. (Rapporteur), P. Andersen, A. Artola, J. Bockaert, T. Bonhoeffer, G.L. Collingridge, M.B. Kennedy, R.C. Malenka, B.L. McNaughton, H. Monyer, R.A. Nicoll and C.F. Stevens "Dahlem Workshop on Cellular and Molecular Mechanisms Underlying Higher Neural Functions (contribution: Group Report: How adequate are current explanations of long-term potentiation/depression?)." Edited by: Selverston, A.I. and P. Ascher, John Wiley & Sons, New York, pp 223-245, 1994.

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Reduced Gravity: Effects in the Developing Nervous System

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FY 1995 Funding: $ Students Funded Under Research: 0

Joint Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Ames Research Center

Task Description:

It is proposed to examine the short-term, intermediate-term and long-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); 2) neuronal migration (i.e., the rate of movement and attainment of proper position; and 3) neuronal differentiation (i.e., the proper orientation of dendrites and axons and the attainment of appropriate neuronal circuitry). 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 or rats) 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. The long-term effects will be assessed by administering the markers and sacrificing after several weeks of survival (after reentry). For these studies, the focus will be on the development of the cerebral cortex, which is a well-studied structure and for which there is a great deal known about normal development. 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. For long-term studies, the final position of cells "born" at particular ages will be analyzed. It is planned to collect brains from specimens of different ages through about 30 days after birth (*i.e.*, P14) at the Kennedy

Space Center. No multigeneration studies are planned. Ideally, experiments would be performed on CD-1 mice for which there already is a great deal of data from other NIH supported projects; however, the experiments could also be performed on rats if the needs of the other participants in Neurolab would require this.

No additional data was provided by the investigator for this research.

Role of Visual Cues in Spatial Orientation

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Initial Funding Date:

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Students Funded Under Research: 2

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Johnson Space Center

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" & "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 3-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" 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 proposal describes 3 new experiments, based on existing 1-G paradigms, which are designed to better define ego-/exocentric

sensory transformations in 0-G; to understand how exocentric frame of reference affects recognition of visual objects; and 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 linear vection") 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.

Our proposal was selected for definition in May 1994. We then worked with others involved in the Sensory Motor and Performance Group on the mission to develop an integrated proposal, which was reviewed and accepted in March 1995. Since then, Dr. Oman has been participating in Investigator Working Group meetings.

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 "Star Tours"; 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 the Neural studies of interaction between visual, vestibular, and proprioceptive orientation cues in 0-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. The laboratories of all three investigators for this experiment (Oman, Howard, and Carpenter-Smith) are currently engaged in the study of the role of vision in a variety of both real and virtual environments. 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 to better understand the role of gravity in the fundamental sensory, motor, and cognitive mechanisms which normally subserve 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 their balance and orientation, and for improving balance function via rehabilitative training. Portable head mounted displays, akin to those used in this Neural experiment, may well prove useful for such testing and training, and perhaps someday even as visual prostheses for vestibularly impaired patients.

Effects of Microgravity on Neuromuscular Development

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Funding:

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Joint Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 aberrances. 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.

Efforts have been made to adapt ground-based standard, laboratory techniques to work under the constraints of the Spacelab facilities and the microgravity environment. Prolonged immersion fixation and 2 weeks of refrigerated storage of fixed muscles was found to be compatible with histochemical staining of neuromuscular junctions. Room temperature storage was unacceptable, necessitating use of

the Spacelab refrigerator. In the process of modifying the endplate staining procedure, an improved staining method utilizing UV light photoactivation developed which will benefit Earth-based studies utilizing the technique. Quick freezing on orbit at liquid nitrogen temperature appears feasible when excised tissues are wrapped in aluminum foil and placed into dry nitrogen shippers. The cold temperature holding time of the commercial unit was insufficient for a 16 day Neurolab mission. Other units will be tested or the existing unit modified for a longer holding capacity. A one way valve to prevent frozen muscles from floating out of the dewar in microgravity will be designed and tested. A stage was designed and tested for video and still photography recording of neonatal rat postflight movements and health status. A prototype microinjection syringe was developed for injecting Nuclear Yellow into neonate muscles to retrograde label soleus and EDL muscle motor neurons. The prototype delivers 1 ml quanta of dye into the muscle. NASA engineers are developing a flight unit for testing. Spinal cord preservation is best accomplished on Earth by whole rat perfusion with warm buffered saline followed by cold aldehyde fixative solution. Adapting this procedure to microgravity presents problems of fixative containment. Alternative procedures were tested which consisted of saline only perfusion followed by immersion fixative in flight approved fix bags. A method of pressure injection of saline was developed to quickly remove the spinal cord from the vertebral column for immersion fixation. Anesthetic concentrations and delivery methods were tested for different aged neonates for definition of appropriate anesthesia for muscle injection survival surgery and perfusion euthanasia.

Future work will involve continued preparations for flight and examination of the effects of simulated flight by hindlimb suspension unloading experiments. The tasks will include completion of analysis of NIH.R3 tissues, studies of the effects of retrograde motoneuron labeling on motoneuron metabolic properties, examination of hindlimb suspension unloading effects on the neuromuscular systems of 8-day-old rats, further definition of inflight animal processing protocols, and testing of prototype flight hardware.

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.

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Autonomic Neurophysiology in Microgravity

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Students Funded Under Research: 4

Joint Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 carotid baroreflex loading and unloading with a neck chamber and skeletal muscle afferent stimulation with isometric and isotonic forearm exercise. Sympathetic baroreflex function will be tested with pharmacologic stimuli (phenylephrine and nitroprusside). We will also determine, in these same subjects, the number and affinity of beta-adrenergic receptors on lymphocytes and alpha2-adrenergic receptors on platelets before and during exposure to microgravity. We will utilize isoproterenol and phenylephrine to quantitate the sensitivity of alpha1 and beta-adrenoreceptor function. 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.

Our main objectives remain unaltered. However, we have worked in the past year to modify the methodology included in our original proposal. This was necessary to accommodate for operational requirements and scientific integration with other Neurolab research studies. The state-of-the-art techniques used to assess sympathetic function remain the same. We will continue to use them to determine the effect of microgravity on "resting" sympathetic function and baroreflex function. In the past year we have altered the stimuli that will be used to unload the baroreflex.

Previous studies, however, have only assessed the parasympathetic limb of the baroreflex. It is not known, therefore, if similar alterations in the sympathetic limb of the baroreflex occur. We will assess this using phenylephrine to load, and nitroprusside to unload arterial baroreceptors. We will now use relatively low levels of lower body negative pressure (LBNP) to unload high and low pressure baroreceptors. This approach has the advantage of simulating orthostatic stress in flight.

For this purpose, we have evaluated an LBNP chamber developed by Dr. Friedhelm Baisch. Initial results indicate the feasibility of using this chamber to perform microneurography during LBNP in space.

An important operation consideration in Neurolab is the potential conflict between experiments. It is possible that studies performed by other investigators may affect autonomic function and, therefore, confound our results. For example, vestibular stimulation is an important scientific component to Neurolab, but its effect on autonomic function is not well understood. We have recently determined that selective stimulation of vestibular afferents, using cold irrigation of the ear, does not alter sympathetic nerve activity or plasma catecholamines. Further studies are under way to assess this and other potential interactions.

The results of these studies will improve our understanding of autonomic mechanisms that regulate blood pressure. It is hoped 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 and, therefore, treatment remains inadequate. We believe the knowledge gained by the Neurolab experiments will help improve the treatment of these patients.

Publications, Presentations, and Other Accomplishments:

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Multidisciplinary Studies of Neural Plasticity in Space

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Funding:

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Solicitation: 93 OLMSA-01

Initial Funding Date:

Expiration:

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Students Funded Under Research: 2

Joint Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 simulate functional interpretations of the integrated 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 micro-g on the SLS-1 mission conserves functionality by increasing hair cell synaptic efficacy, particularly in the intrinsic distributed modifying microcircuit. 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 whether otolith organ plasticity includes changes in otoconial mass; 3) to correlate the anatomical and physiological findings with results of studies aimed at uncovering the molecular basis of macular synaptic plasticity; and 4) to interpret the functional significance of the macular microcircuits through computer simulations 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 research will electrophysiologically characterize the changes in response properties of vestibular afferents of one set of rats, using multichannel electrodes chronically implanted in Scarpa's ganglion, as the gravitational environment varies between pre-, in-, and post-flight conditions. A separate pool of rats of similar

ages derived from the same genetic pool will be used to collect anatomical evidence of structural changes and to sort out the molecular basis of macular synaptic plasticity.

Data from SLS-2 continued to be analyzed in preparation for Neurolab, and three-dimensional (3-D) reconstructions of flight and control tissue were begun in FY95. Gathering synapse data from utricular gravity sensors of flight and control rats used for SLS-2 is taking longer than for SLS-1. This is due to the need to use 100 sections for 3-D reconstructions of hair cells and innervation patterns. However, use of this many sections is resulting in exceptionally reliable data since means of synapses from animal to animal in a specific group (such as inflight or ground control) match very well. The most important finding is a doubling of synapses in type II sensory cells in-flight compared to controls, with a rapid subsidence to lower counts post-flight. Results are, in general, matching the trends established in SLS-1. Also, we have completed six 3-D reconstructions of various cells, calyces and innervations. These reconstructions are revealing new details of the innervation of gravity receptors not previously known, such as the regularity of a nerve fiber process innervating two type II cells, multiple processes from a calyx to a type II cell, and a broader area of receptive field overlap than realized by our previous mappings. The use of every section for the reconstructions has made the difference. The work shows the practicality of the software we have developed for 3-D reconstruction which will be used for Neurolab results. In addition, a number of preparatory procedures for studies of electrophysiological responses to exposure to altered gravity (in this case, hypergravity) have been carried out. The precise location of Scarpa's ganglion was determined and all surgical procedures were completely tested. A new multichannel electrode using a chip design has been developed for this specific experiment, so that several neurons can be recorded from simultaneously. The progress made indicates that we shall be well prepared for utilization of experimental, analytical and computer-aided reconstruction tools developed during the preparatory period for Neurolab.

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. At the same time, 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. In addition, the software developed for 3-D reconstruction of neurons and innervation patterns in gravity sensors has numerous ramifications. First of all, it permits the wiring pattern of a simple neuronal system to be unraveled for scientific study and simulation. This will mean that, 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 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 co-journiers on the space vehicle. Virtual laboratories will permit training before necessary intervention takes place.

Publications, Presentations, and Other Accomplishments:

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Ross, M.D. "Mammalian Vestibular Macular Synaptic Plasticity: Results From SLS-2 (Space Life Sciences-2) Space Flight." ARO Midwinter Meeting, St. Petersburg Beach, FL, February 5-9, 1995.

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The Stress of Space Flight: Effects on Learning

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Funding:

Project Identification: E052

Solicitation: 93 OLMSA-01

Initial Funding Date: 7/95

Expiration: 12/99

FY 1995 Funding: $351,278

Students Funded Under Research: 6

Flight Information:

Flight Assignment: NIH-H (STS-85, 1997)

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 effects 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 inflight 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 have met to assemble and test the project equipment and work on the integration of the computer hardware and software. Parameters for the non-associative and associative conditioning

paradigms with the new equipment are being determined in order for the ground-based control subject tests to be conducted in the remainder of FY 1995.

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 posttraumatic stress disorder), the results of these studies could indicate how stressor intensity contributes to these disease processes.

Publications, Presentations, and Other Accomplishments:

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Effects of Microgravity on Postnatal Motor Development

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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding:

Students Funded Under Research:

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Ames Research Center

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 proposed 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 (swimming, 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. Preflight 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). Inflight 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.

Our efforts these last few months have been to determine if nursing rat neonates will survive under the condition encountered on a Shuttle flight. After establishing the ages of animals that would be tested on a mission, we participated at experiments at Ames Research Center to establish the optimal preflight procedure to select dams and neonates that would comprise the flight and ground control animals. Using such animals, the design of the cages were tested on STS-72 in January, 1996. The ages of the animals at launch were postnatal day 5, 7 and 14.

The primary role of our lab was to use behavioral measures of motor system development to find if our Neurolab specific objectives could be accomplished. The answer is yes. Preliminary data was gathered at Hanger L at Kennedy Space Center from the landing day (R+0) to R+14. The animals were shipped to New York University on 2/5/96. Motor functions were assessed everyday (R+0-R+14) by videotaping the animals swimming, walking, surface righting, and air righting. Other, more complex tests were carried out on selected days. These data are being analyzed now and indicate a difference between flight and ground controls.

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 bed rest.

Sleep and Respiration in Microgravity

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Funding:

Project Identification: E198 Solicitation: 93 OLMSA-01

Initial Funding Date: 10/94 Expiration:

FY 1995 Funding: $46,373 Students Funded Under Research: 1

Joint Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

Responsible NASA Center: Johnson Space Center

Task Description:

There is evidence that sleep is affected by microgravity (μg). However, despite anecdotal reports of poor quality of sleep in μg, 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, 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 μg

We propose to 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 with an ECG. From these sensors, we can determine changes in ventilation, relative rib cage and abdominal contribution to ventilation, thoraco-abdominal asychrony, 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 propose to 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. Pre- and post-flight, 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.

Integration of our original proposal with that of Dr. Charles Czeisler from Brigham and Women's Hospital, Harvard Medical School has occurred! This resulted in an integrated proposal and protocol for the Sleep Team being submitted.

The joint Experiment Document has been developed and submitted and a Preliminary Design Review was held. Hardware development for the Digital Sleep recorder and for the Isocapnic Hypoxic Rebreathing Critical Design Review for both items is scheduled for May.

Sleep is often poor in μg 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.

Publications, Presentations, and Other Accomplishments:

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Development of Vestibular Organs in Microgravity

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Solicitation: 93 OLMSA-01

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Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Joint Participation: National Science Foundation

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)

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 stones 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 C.E.B.A.S. system. After recovery, some specimens will be fixed for light and electron-microscopic examination. The statolith and 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.

We have established breeding colonies of two strains of the water snail *Biomphalaria glabrata* in our laboratory. Ground-control studies of the development of the statocyst are underway. The basic structure of the *Biomphalaria* statocyst has been elucidated. In some respects, the statocyst resembles that in the marine mollusk, *Aplysia*, with which we have previously worked. As in *Aplysia*, the statocyst contains thirteen ciliated receptor cells and numerous supporting cells. The statoconia are produced in the supporting cells and exocitosed into the cyst lumen. Different from *Aplysia*, in *Biomphalaria*, the embryonic statocyst, contains multiple statoconia before the animals hatch. No single statolith, as seen for the first 40 to 60 days in *Aplysia*, has yet been identified in *Biomphalaria*. We are currently compiling statistics on the increase in number and volume of statoconia as the animal grows. Electron-microscopic study of the statocyst has also begun. This has shown that the cellular features in *Biomphalaria* are quite similar to those in the *Aplysia* statocyst. However the clear laminar structure seen in *Aplysia* statoconia has not been seen in *Biomphalaria*.

We have also begun analysis of crawling tracks of juvenile and adult *Biomphalaria*. These studies are currently being done manually. If they demonstrate a preferential direction of crawling relative to gravity, we will construct the rotating platform to study gravitactic behavior and obtain software to automatically analyze crawling tracks.

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 ability to develop gravity-related reflexes in the absence of gravity.

Publications, Presentations, and Other Accomplishments:

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Pedrozo, H.A., Z. Schwartz and M.L. Wiederhold "Aplysiastatocyst in culture: A novel organ culture system." Amer. Soc. for Gravitational and Space Biology, Bulletin 8, no 1, 7 (1994).

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Inflight Radiation Measurements

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Funding:

Project Identification: 5.2.1

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

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Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The United States and Russian space agencies use different equipment to measure the levels of radiation within their spacecraft. By comparing independent space radiation dose calculations and radiation level measurements, this experiment provides a way to verify the dosimetry procedures of each space agency, to identify any potential systematic errors, and to increase the database of information available for analyzing and modeling changes in the radiation environment.

One of the objectives of this experiment is to simultaneously take independent measurements of space radiation exposure using US hardware and Russian hardware and compare the two measurements. Another objective is to provide more accurate mapping of the South Atlantic Anomaly (this is a region over the South Atlantic which has a high concentration of radiation). A third objective is to compare the measured linear energy transfer spectra for the galactic cosmic ray (GCR) and trapped proton components with the calculated spectra for those sources.

To accomplish these objectives, crew members wear passive dosimeters (one American device and one Russian device) to measure the radiation levels to which they are exposed. They also place various radiation measurement devices (one from each country) throughout Mir and the Shuttle.

No additional data was provided by the investigator for this research.

Anticipatory Postural Activity During Long-Duration Space Flight

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Project Identification: 4.2.4

Solicitation: US/RSA Negotiations

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Students Funded Under Research: 14

Flight Information:

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

Responsible NASA Center: Johnson Space Center

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 and Mir 19. Data analysis is proceeding steadily and several preliminary reports have been made. The results are corresponding to the hypotheses.

The project has flown aboard STS-63, STS-71 Mir 18, Mir 19 and is scheduled to be performed aboard Mir 21. To date 5 subjects have completed the inflight portion of the experiment and 8 subjects have completed the ground-based experimental conditions.

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.
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 integration of the area under the EMG curve, measures of muscle co-contraction have also 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 integration of the area under the EMG curve, measures of muscle co-contraction have also been developed.
c) center of pressure (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 in 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 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 dynamical computational model of a human is in development (in conjunction with investigators at the University of Texas at

Austin). This model uses algorithms which enable it to predict optimal solutions to a variety of movement tasks.

So far, we have encountered no anticipated questions. 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.

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 achieve 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 raining protocols.

Publications, Presentations, and Other Accomplishments:

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Layne, C.S., McDonald, P.V., Pruett, C.J., Jones, G., and Bloomberg, J.J. "Preparatory postural control after space flight." Society for Neuroscience Annual Meeting, San Diego, CA, November, 1995.

Pruett, C.J., Layne, C.S., Jones, G., Bloomberg, J.J., McDonald, P.V. "A foot-pressure device for neuromuscular applications." National Conference of the American Institute of Aeronautics and Astronautics, Houston, TX, April, 1995.

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

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Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Locomotor disturbances frequently occur following space flight and have been reported by both U.S. and Russian space programs. During space flight, neural adaptive processes come into play recalibrating the central nervous system 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 on return to Earth.

During locomotion, angular head movements act in a compensatory fashion to oppose vertical trunk translation that occurs during each step in the gait cycle. This coordinated strategy between head and trunk motion serves to aid gaze stabilization and perhaps simplifies the sensory coordinate transformation between the head and trunk allowing efficient descending motor control during locomotion.

The aim of the present study was to determine if eye-head-trunk coordination strategies, that occur during terrestrial locomotion, are modified following long-duration space flight and ascertain if these changes are associated with disturbances in gaze control, lower limb kinematics and muscle activity patterns of the leg during locomotion. Obtaining this information will aid in the design and evaluation of sensory-motor countermeasures against the deleterious effects of long-duration space flight.

The general objectives of this investigation were to:
• Determine if exposure to the microgravity environment encountered during space flight adaptively modifies eye and head control mechanisms required to maintain gaze stability during terrestrial locomotion.
• Determine if head-trunk coordination strategies, that occur during terrestrial locomotion, are modified following space flight and determine if these changes are associated with disturbances in lower limb kinematics and muscle activity patterns of the leg during locomotion.
• Subjects were asked to walk and run on a motorized treadmill while fixating their gaze on an Earth-fixed target. The ocular target was located either 2m or 30 cm from the eyes to characterize changes in strategy associated with different goal-directed gaze tasks.

During performance of all these tasks head and body kinematic data were collected with a video-based motion analyzing system. Eye movements were recorded using standard DC electro-oculographic (EOG) methods while surface electromyographic (EMG) methods were used to characterize muscle activity patterns of the leg.

Mir-18 pre and postflight data were collected on three subjects. Three preflight (120, 45, and 10 days before launch) and five postflight (1, 4, 6, 9, 12 days after landing) data collections were performed. Two preflight collections took place in Star City, Russia and all other data collections occurred at the Johnson Space Center.

All pre and postflight video data have been processed through the following steps of data reduction: 1) Tape Dumping: the process of replaying the tapes through a video processor and outputting an individual digital file for each of four cameras; 2) Tracking: calculating 3-dimensional trajectories of each body-fixed marker using the computer-based algorithms and camera calibration procedures; 3) File Transfer: converting data to analyzable MATLAB format and transferring the 3-D trajectory output files and electrooculography data to a computer network; this includes renaming trajectory variables and synchronizing EOG analog data with trajectory traces; 4) Fourier spectral analysis of compensatory head movements; and 5) Analysis of lower limb muscle activation patterning.

Several preliminary observations were made. Crew members show significant alterations in head-trunk coordination following long-duration space flight. Also, the Mir-18 crew show disruption in lower limb muscle activation patterns during locomotion that exceeds that shown by Shuttle crew members. Finally, microgravity induced alterations in head-trunk coordination during locomotion may play a central role in astronaut locomotor dysfunction that occurs following space flight.

This investigation is one component of an integrated program of Neuroscience experiments being conducted at the Johnson Space Center designed to examine microgravity induced adaptive modification of spatial orientation and motion perception processes, gaze control mechanisms, 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 aged 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 of 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 flights of longer duration we may find changes that are not 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 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.

Publications, Presentations, and Other Accomplishments:

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Bloomberg, J.J., Huebner, W., Layne, C., McDonald, P., Reschke, M., Peters, B., and Smith, S. "Evaluation of microgravity induced adaptive modifications in sensory-motor function." American Institute of Aeronautics and Astronautics, Life Sciences and Space Medicine Conference, Houston, TX, April 3-5, 1995. Abstract in AIAA Book of Abstracts, p. 22-23.

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Physiological Response During Descent on the Space Shuttle

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Funding:

Project Identification: 3.3.1

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The objective of this experiment is to document the changes in blood pressure, heart rate, and other physiologic parameters during the Shuttle landing. This data will be related to the mission duration and changes in orthostatic function of crew members during entry, landing, and exiting the Shuttle.

During the last hours in flight, the Mir-18 crew members are connected to instruments for monitoring during pre-landing, landing, and post-landing periods. Unlike the rest of the shuttle crew, the three Mir-18 crew members return to Earth lying down. After landing, they perform a stand test. The results from this study will be used to evaluate the effectiveness of proposed countermeasures used during flight by determining whether orthostatic function is improved. These data will also be used to determine whether additional precautions and countermeasures are needed to protect crew members in the event of an emergency.

Studies on Orthostatic Tolerance with the Use of LBNP

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Funding:

Project Identification: 3.1.1

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

A major concern of scientists for the process of cardiovascular adaptation is *post flight orthostatic intolerance*, which can be defined as the difficulty a crew member may experience when standing upright upon return to Earth due to the cardiovascular system's inadequate blood flow to the brain. Orthostatic intolerance is a safety concern since crew members are in an upright posture during Shuttle reentry and landing. On some missions, 60% of crew members could not complete a stand test after landing. Current theory holds that the decrease in blood volume, the change in reflex control of blood pressure, and the pooling of blood in the legs and abdomen are major contributing factors to this post flight orthostatic intolerance. During the three-month Mir mission, there may be a significant loss of orthostatic tolerance by the end of the first month in orbit, after which no further significant loss in orthostatic tolerance is expected

The objective of this experiment is to uncover the mechanisms involved in the reduction in orthostatic tolerance. Medical investigators measure the in-flight orthostatic tolerance of crew members using the Russian device for lower body decompression, the "Chibis," while on Mir, and the American Lower Body Negative Pressure (LBNP) device after transferring to the Shuttle. These devices are used to decompress the lower part of the body (pulling fluid into the abdomen and legs), while various cardiovascular parameters, such as heart rate, arterial blood pressure, and cardiac dimensions, are measured using the American Echocardiograph Research Imaging System (AERIS) and other equipment. The results from the in-flight study are compared to the results obtained before and after flight as well as to results from similar studies performed on Shuttle astronauts on shorter flights.

No additional data was provided by the investigator for this research.

Morphological, Histochemical, and Ultrastructural Characteristics of Skeletal Muscle

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Funding:

Project Identification: 4.1.2

Solicitation: SMSP Phase 1A

Initial Funding Date: 1993

Expiration:

FY 1995 Funding: $77,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Skeletal muscle strength and lower limb girth measurements of Skylab and Apollo crew members indicated significant loss of muscle mass as a result of exposure to the microgravity conditions of space flight. Recent investigations using magnetic resonance imaging (MRI) to determine muscle volumes of selected skeletal muscles and muscle groups in Shuttle crew members revealed that small muscle volume decrements (in the range of 4 to 10%) occur even in short duration missions in spite of current inflight exercise protocols. This task was undertaken to measure muscle volumes in muscles and muscle groups shown during short-duration Shuttle flights to be most prone to space flight-associated skeletal muscle atrophy in a long-duration (115 day) stay aboard the Russian Space Station Mir. Muscle volumes were determined by MRI in the leg (soleus, gastrocnemius, anterolateral compartment), thigh (hamstrings, quadriceps), intrinsic lower back (rotatores, multifidus, spinalis, longissimus, iliocostalis) and psoas, and posterior neck of all crew members on this long duration mission. All imaging was performed on a 1.5 Tesla Siemens magnet using spine or whole body coils. Imaging was performed on two occasions prior to flight at approximately 149 and 32 days prior to launch and 2-3 days after landing. For the thigh and calf muscles, thirty-two slices of 1 cm thickness were obtained using a Te=22 msec, Tr=1.5 sec and 256x256 matrix. For back muscles, an imaging spine coil was employed and twenty 0.5 cm thick slices centers on L3 were obtained using a Te=20 msec and Tr=1.0 sec. A phantom was imaged with each session to assure consistent pixel size. Matching of extremity slices from one imaging session to the next was accomplished by comparing the bone marrow areas. The matching of back muscle image slices was accomplished by using a position matching program on sagittal slices from each imaging session. The volume of muscle tissue in each slice was determined in a semi-automatic method using Sunvision software. The two preflight measurements were averaged and the mean ±SD percent change from preflight was calculated for each

muscle or muscle group. Student's *t*-test was used to determine significance at the $p<0.05$ level. Volumes of all muscles and muscle groups were significantly decreased except for the psoas. Mean percent changes were as follows: soleus, -18±4%; gastrocnemius -18±1%; anterolateral calf, -15±3%; hamstrings, -13±4%; quadriceps, -11±3%; intrinsic lower back, -18±45; and psoas -6±6%. In addition to MRI, venous blood samples were obtained before, during, and after flight for analyses of creatine kinase activity and myoglobin to determine if evidence of muscle damage was present during the course of skeletal muscle atrophy.

These results document the extent of muscle volume changes that occurred in 3 crew members during a 115 day space flight. Although exercise was performed during the flight that varied by individual crew member, the degree of volume loss that occurred was similar for each. Comparison of the amount of muscle volume decrements measured during a short duration Shuttle flight with that of this flight suggests that the rate of loss of skeletal muscle mass is nonlinear which agrees with ground-based studies using short and long term bed rest as a simulation of microgravity associated muscle unloading. Compared to bed rest of similar duration (117 days), this 115 day flight resulted in less atrophy in the soleus/gastrocnemius and quadriceps but more atrophy in the intrinsic lower back muscles. It appears that MRI volume assessment of skeletal muscle is an informative, noninvasive method to monitor both current and future muscle atrophy countermeasures and to provide clinically useful information for the design of individualized postflight rehabilitation plans following long duration space flight.

Loss of muscle mass occurs in a variety of conditions that affect Earth-bound humans. These range from the atrophy associated with cast-immobilization following traumatic injury to primary or secondary genetic diseases affecting skeletal muscle or its innervation. Any medical condition resulting in bed rest and loss of the daily muscle activity that occurs against normal gravitational resistance may result in various degrees of muscle loss similar to that experienced in space flight. Countermeasures that are developed to offset the muscle loss associated with space flight have potential in attenuating muscle atrophy that occurs in a variety of medical conditions found on Earth. This task has proven the benefit of MRI muscle volume measurements in assessing the degree of muscle tissue loss and can be used similarly on the ground to validate clinical interventions that may arise from positive results obtained in ameliorating space flight associated muscle atrophy.

Evaluation of Thermoregulation During Long Duration Spaceflight

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Funding:

Project Identification: 3.2.2

Solicitation: US/RSA Negotiations

Initial Funding Date: 1995

Expiration: 1996

FY 1995 Funding: $40,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Impaired thermoregulation, which has been observed during exercise following bed rest, 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 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/°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 $mg*min-1*cm^{-2}/°C$) 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.

A. Objectives of the Experiment

1. Determine whether overall thermoregulation is impaired during space flight by comparing core temperature during two submaximal levels of aerobic exercise, preflight, in-flight, and postflight,
2. Determine whether the elevated core temperature is due to altered heat production, as calculated by indirect calorimetry,
3. Determine whether the elevated core temperature is due to impaired heat loss, as assessed by the skin blood flow/core temperature relationship (where changes in skin blood flow are measured pre- and postflight by laser Doppler flowmetry, and estimated during all tests by calculations of tissue conductance) and by the sweating response (local sweat response/core temperature relationship are measured pre- and postflight using a dew point hygrometer, and total body sweat loss is estimated during all tests by measuring body weight loss.

B. Methods/Accomplishments to date.

Submaximal exercise tests were performed in the two subjects preflight (approximately L-145) and as soon as possible postflight (L+5). There will be no further opportunities for data collection after long duration space flight.

The submaximal exercise tests consisted of 5 minutes of quiet rest, then 20 minutes of exercise at 40% preflight VO_2 peak, followed immediately by 20 minutes of exercise at 65% preflight VO_2 peak, followed by 10 minutes of quiet resting recovery. Heart rates during submaximal exercise were to be monitored with an EKG system built into the MedGraphics Metabolic Gas Analysis System. Blood pressures were to be obtained twice before exercise, approximately every 10 minutes during exercise, and twice after exercise with a manual sphygmomanometer. Skin temperatures were to be measured continuously with four external skin thermistors (forearm, calf, thigh, chest) and recorded with a "Squirrel Data Acquisition System." Core temperature was to be measured by ingesting a small telemetry thermal sensor pill (Human Technologies Inc., HTI) 6 hours before exercise and data sampled every 30 seconds and stored in the HTI receiver. Heat production was to be calculated from VO_2 measurements obtained before exercise and once every 10 minutes during exercise. Cardiac output measurements (CO_2 rebreathing) were to be obtained at rest before exercise and twice at each exercise level. Total body sweat loss was to be assessed from changes in body weight obtained immediately before and after exercise, using a standard scale preflight and the body mass measurement device in flight. Skin blood flow was measured continuously during the submaximal exercise test with a laser Doppler skin blood flow sensor on the arm next to the skin thermistor (Primed P4 system with an integrated laser probe). Local sweating from a chest site was measured continuously using a dew point hygrometry sweat system.

C. Results

Exercise time was shortened in both crew members postflight. In each postflight test, the crew member was told to stop exercise by the flight surgeon due to concern about the crew members elevated heart rate response compared to preflight.

The sensitivity of the sweating response (slope of the sweat rate/core temperature response) was reduced in both crew members on landing day compared to preflight. The onset of sweating, the threshold, was shifted to a higher core temperature in one crew member but not appreciably changed in the second crew member.

The sensitivity of the skin blood flow response (slope of the skin blood flow/core temperature response) was reduced in both crew members postflight without appreciable change in the threshold for the onset of vasodilation.

D. Discussion

It is too early at this time to make any definitive statements about the findings of this investigation. Further, the small number of subjects will not allow statistical analysis of the data and will result only in case study reports. We recently have been awarded a second grant (NRA solicitation) to collect data from an additional six crew members after short duration Shuttle flights. The addition of these crew members should allow us to obtain a sufficient number of data points to confirm whether skin blood flow and sweating responses are compromised following space flight. In addition, the new grant should allow us to collect inflight data to directly assess the effects of microgravity on thermoregulation. (This was originally an objective of this Shuttle-Mir proposal, but due to flight logistics problems, no inflight data was obtained).

E. Conclusion

For the two crew members there appears to be a trend towards an increased core temperature during exercise accompanied by attenuated skin blood flow and sweating responses postflight when compared to preflight responses. These results suggest that ground based predictions and models of thermoregulation may underestimate the susceptibility of crews to heat stress during egress and following space flight.

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 during ground based conditions in which heat loss responses are impaired (patients with inability to vasodilate appropriately such as hypertension, patients with impaired sweat responses, 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, already countermeasures for heat stress experienced in the space program (eg. liquid cooled garments, EVA suit life support system) have 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.

Assessment of Autonomic and Gastric Function During Spaceflight, Entry and Landing

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The overall goal of this investigation is to enhance our understanding of the effects of adaptation to space flight and readaptation to Earth on autonomic and gastric function. At least some symptoms of motion sickness are experienced early in flight by most crew members and postflight by the majority of crew members returning from long duration missions. Physiologic measures of motion sickness are necessary as objective indicators of symptom severity and occurrence, and as clues to the understanding of the physiological mechanisms involved in the development and resolution of symptoms. This study proposes to evaluate changes in two non-invasive physiological measures, electrogastrography (EGG) and the frequency components of the cardiac interbeat interval (IBI), for this purpose. EGG and cardiac IBI data will be collected preflight under fasted, fed and motion sick conditions; inflight on long duration (90 or 180 days) missions before, during and after performance of head movements to elicit SMS symptoms; during entry and landing; and postflight during the readaptation period. Data will be collected by an ambulatory recording unit which has previously flown on the Shuttle. Changes in the frequency and amplitude of the EGG and in the frequency components of the cardiac IBI are expected to occur with or even before subjective reports of motion sickness symptoms. The characteristics of the changes in frequency of the cardiac IBI and possibly EGG are expected to provide insight into which branch of the autonomic nervous system predominates during SMS symptom development and resolution. Understanding of the physiological mechanisms involved in SMS symptom development would greatly increase our ability to develop effective countermeasures. Additionally, identification of "early warning" indicants of SMS would allow pharmacologic and behavioral countermeasures to be applied early (thus maximizing their effectiveness) and only to those requiring them.

N/A: Descoped from NASA-Mir program.

The goal of this investigation is to better understand changes in gastric activity and autonomic mechanisms involved in space motion sickness. This work should provide similar insights into all types of terrestrial motion sickness. Better understanding of the physiological processes involved in all forms of motion sickness may lead to better, more targeted pharmacological treatments with fewer side effects. Continued development of the technology could lead to a device that could monitor and provide early warning indication of impending motion sickness. This would allow more timely and appropriate treatment (behavioral and/or pharmacologic) interaction.

Trace Chemical Contamination

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Funding:

Project Identification: 5.3

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $242,270

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The investigators for this experiment are studying the characteristics and the dynamics of the atmosphere on Mir and the chemical composition of Mir potable water. In addition to providing a better understanding of each separate system, it will provide a better understanding of the interaction between atmospheric and water contaminants.

To accomplish these goals, several types of sampling devices will be used to collect air and water samples. Instantaneous "air" samples and time-integrated air samples will be collected for ground-based analysis of volatile organic contaminants, carbon monoxide, and hydrogen. Samplers will be placed in specific areas of the spacecraft and will be worn by crew members to determine levels of formaldehyde. To ensure that water being consumed by the Mir crew meets established quality standards, samples of potable water will be collected during the Mir-18 mission for post flight analysis. Humidity condensate samples will also be collected to determine the inter-relationship between air contaminants and water contaminants from atmospheric condensate. Samples will be jointly analyzed by U.S. and Russian laboratories and will focus on inorganic and organic compounds. The information gathered by this research will help scientists and engineers develop and evaluate water purification units, water quality standards, and in flight water sampling hardware and procedures for future space stations.

Toxicology Information - The atmosphere of Mir was evaluated for trace chemical pollutants during expeditions 17, 18, and 19. In general, the atmospheric contaminants were greater than those found in

the Space Shuttle, but the Mir contaminant levels still meet U.S. acceptability standards except for mucosal irritants. In particular, formaldehyde, a mucosal irritant and carcinogen was consistently found in concentrations slightly above the U.S. and Russian limits of 0.04 ppm. Efforts are underway to improve our control of formaldehyde in spacecraft air by limiting sources such as hardware offgassing. Data from Mir 18 indicate that spatial variations in pollution levels are less than 20%. Temporal variations are generally limited unless a new source of air pollution, such as a new module opening or experiment startup, occurs. During Mir 18, a large spike of Freon 82 into the air was noted late in the expedition. Preliminary, data from Mir 19 appear to be quite similar to data from Mir 18. In cooperation with NASA engineers, NASA toxicologists and chemists have developed a thorough plan to continue air sampling and analysis throughout the Shuttle/Mir Program.

Water Information - Chemical analysis of 5 water samples collected during Mir 18 and STS-71 missions have been completed. The results show that the reclaimed water met all Russian Space Agency (RSA) water quality standards, all NASA water-quality standards except for methylene chloride. The total organic carbon levels in the reclaimed water ranged from 1.3 to 6.7 mg/L compared to the NASA limit of 0.5 mg/L. Analysis of Mitischi water (Russian mineralized tap water) sampled on March 6, 1995 and of Mir ground-supplied potable water taken on July 3, 1995 showed total organic carbon levels of 8.7 mg/L and 6.6 mg/L, respectively. Only 5 percent of the organic content in the reclaimed water could be identified using available analytical techniques, indicating the need for further development of analytical methods.

Preliminary sample analysis on four potable water samples (three taken during Mir 19 and one taken during STS-74) and four humidity condensate samples (one taken during Mir 20 and three EDV samples from STS-74) have also been completed. The results of the Mir 19 humidity condensate sample show that the total organic carbon was 18.5 mg/L. Also, this sample contained a high level of methylene chloride (28.3 mg/L). The organic carbon balance indicates that 32.4% of the organic carbon was accounted for in this sample.

The results of the Mir 20 and STS-74 condensate samples indicate the ethylene glycol levels were <2.0 mg/L in the 10/30/95 sample that was collected before the ethylene glycol spill, 149.2 mg/L in the first tank after the spill, 152.8 mg/L in the second EDV tank, and then 44.0 mg/L in the third EDV tank. The total organic carbon concentrations followed a similar pattern starting at 36.8 mg/L in the 10/30/95 sample, increasing to 70.1 in the first tank, 79.8 mg/L in the second EDV tank, and decreasing to 57.9 mg/L in the third EDV tank.

The results of the regenerated hot water samples taken during Mir 19 indicate that the TOC levels ranged from 1.48 to 2.86 mg/L. Phenol was detected in these samples at concentrations above the NASA maximum contaminant level of 1 ug/L for total phenols. The concentrations of phenol in the hot water samples ranged from 5.7 ug/L to 6 ug/L.

The results for the STS-74 SVO-ZV (ground supplied) water sample indicates the TOC levels was 6.99 mg/L. This sample contained significant levels of acetaldehyde (3,034 ug/L), acetone (126 ug/L), and chloroform (205 ug/L). This chloroform level is above the U.S. EPA maximum contaminant level of 100 ug/L.

The air sampling devices developed and employed by NASA, in particular, the Solid Sorbent Air Sampler, can have practical applications for sampling closed spaces. For example, we have been discussing air sampling in submarines and commercial aircraft with the U.S. Navy and Federal Aviation Administration, respectively. Health effects may result from air pollution in these confined spaces.

This research will provide benefits in the areas of methods development for the analysis of drinking water, advanced technologies for the treatment of waste waters, 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 nearly all organic constituents, even those at extremely low levels. In addition, by adapting techniques for treating spacecraft waters, the development of better waste water treatment technologies on earth will be supported.

Publications, Presentations, and Other Accomplishments:

Homan, M.H., Mudgett, P.D., Schultz, J.R., and Sauer, R.L. "GC/MS and CE methods for the analysis of trace organic acids in reclaimed water supplies." Proceedings of the 24th International Conference on Environmental Systems, SAE #941392, Friedrichshafen, Germany, July 1994.

Straub II, J.E., Schultz, J.R., Michalek, W.F., and Sauer, R.L. "Further characterization and multifiltration treatment of Shuttle humidity condensate." Proceedings of the 25th International Conference on Environmental Systems, SAE #951685, San Diego, CA, July 1995.

Dynamics of Calcium Metabolism and Bone Tissue

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Funding:

Project Identification: 2.1.2

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $240,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The effect of space flight on the skeletal system is one of the most critical issues which needs to be resolved to assure crew well-being during extended duration missions. Due to the absence of weight bearing loads and other factors, bone mineral is lost during space flight. As early as flight day two of the SLS-1 mission, serum ionized calcium concentrations were elevated 40% above preflight levels, indicating a change in the calcium balance of the body. Decreased levels of parathyroid hormone and other regulatory factors were also noted. In addition, crew members of Skylab missions were observed to experience calcium loss.

These studies are designed to provide information on the causes of, and possible countermeasures for, the microgravity-induced loss of bone mass. Calcium absorption and kinetics are determined before, during, and after the mission, enabling investigators to understand the impact of dietary calcium on bone loss. Other related measurements include monitoring bone density, bone and calcium regulating hormones, and urinary markers of bone metabolism.

This mission also marks the first time that crew members measure blood pH and ionized calcium concentrations during flight. These measurements will provide significant information for scientific understanding of the effects of space flight on bone calcium. Post flight studies are designed to examine the recovery of bone mineral lost during the mission, as well as readaptation of bone-regulating hormones and calcium metabolism. Potential benefits of this research include further understanding of the countermeasures required for extended duration space flight as well as the potential impact on treatment of skeletal disorders in the general population.

The 30-day and 180-day reports have been generated and submitted. Data have been reviewed and provided to Russian counterparts. Data were presented at the Mir 18 data sharing meeting in Houston, the NASA/AIAA Life Sciences Space Medicine conference in Houston, and will be presented at the Experimental Biology meeting in April.

Methods were developed to study the absorption of calcium from the diet during space flight. These methods are significant improvements over those commonly used in nutrition research in that: the doses are almost 100x lower which will result in decreased cost, and the use of saliva samples will reduce blood requirements.

Fluid and Electrolyte Homostasis and its Regulation

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Funding:

Project Identification: 2.1.1

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $240,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Exposure to microgravity is known to have profound effects on fluid balance. The head ward shift of fluids observed in microgravity results in increased excretion of fluids and electrolytes. In addition to this loss of total body water, the manner in which fluid is stored changes, both inside and outside of individual cells. Although the short-term effects of microgravity on fluid and electrolyte balance have been studied, the effects of prolonged exposure on this set of systems have not been well defined. Determining the nature and extent of fluid/electrolyte loss, as well as the physiological processes of adaptation to microgravity, is required for the development of countermeasures for future extended duration missions.

Fluid and electrolyte balance in the body is regulated by several systems, any or all of which are potentially responsible for the microgravity-induced changes in fluid balance. The kidneys play an important role in the regulation of fluid and electrolyte excretion and/or retention, and it is likely that changes in renal blood flow are important in the adaptation to space flight. There are many endocrine and circulatory factors which regulate fluid homeostasis, both in conjunction with and independent of the renal system. Dietary intake affects fluid and electrolyte homeostasis and may also affect the ability of the body to adapt to microgravity.

This experiment is designed to study the nature and extent of fluid shift and/or loss during an extended duration mission aboard the Mir space station, specifically through investigation of possible effects and interactions of kidney, circulatory, and hormonal influences on fluid and electrolyte balance in microgravity. The information gained from these studies will be important in understanding the body's regulatory system, both during space flight and here on Earth.

The 30-day and 180-day reports have been generated and submitted. Data have been reviewed and provided to Russian counterparts. Data were presented at the Mir 18 data sharing meeting in Houston, the NASA/AIAA Life Sciences Space Medicine conference in Houston, and will be presented at the Experimental Biology meeting in April.

Non-radioactive methods were developed to study extracellular fluid volume. This will assist ground-based studies of disorders of fluid distribution in groups where the use of radioisotopes is prohibited - e.g., children, pregnant women, and the elderly, as well as studies of the physiological effects of physical activity.

Red Blood Cell Mass and Survival

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Funding:

Project Identification: 2.2.5

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $240,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The objective of this experiment is to determine the effects of long-term exposure to weightlessness on RBC production. It is believed that fluid redistribution, followed by the loss of blood plasma volume, results in a higher concentration of RBCs. The body perceives this higher concentration as an excess number of RBCs and appears to decrease their production. To test this hypothesis, the mass of RBCs is measured before, during, and after extended exposure to microgravity. In addition, the life span of RBCs is measured before and after space flight. The hormone erythropoietin, responsible for stimulating RBC production, is also measured.

Before and after flight, blood samples are collected and labeled with a stable isotope. The tagged RBCs are then reinfused into the subject. Follow-up blood samples are collected a short time later.

Investigators who have studied this phenomenon have proposed a new hypothesis that red blood cells are still being produced in the bone marrow, but are not being released as mature blood cells. Rather, they die prematurely. If these results can be confirmed, scientists will reexamine their understanding of red blood cell production here on Earth.

The 30-day and 180-day reports have been generated and submitted. Data have been reviewed and provided to Russian counterparts. Data were presented at the Mir 18 data sharing meeting in Houston, the NASA/AIAA Life Sciences Space Medicine conference in Houston, and will be presented at the Experimental Biology meeting in April.

Non-radioactive methods were developed to study the amount of red blood cells in the body. This will assist ground-based studies of blood disorders in groups where the use of radioisotopes is prohibited- e.g., children, pregnant women, and the elderly, as well as studies of the effects of altitude on red blood cell metabolism.

Alterations in Postural Equilibrium Control Associated with Long Duration Space Flight

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Project Identification:

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The sensorimotor systems of humans have evolved to optimize body movements and posture control in the terrestrial gravitational field. The central nervous system (CNS) has developed neurosensory systems that monitor and process sensory inputs from visual, vestibular, somatosensory, and proprioceptive receptors to assess the biomechanical state of the body (spatial orientation), and neuromotor systems that create, select, and employ motor command strategies and synergies to adjust the biomechanical state toward the desired equilibrium point. Adaptation to microgravity alters neurosensory systems by eliminating, reinterpreting, or modifying the weighting of sensory information used to assess spatial orientation in response to the sudden loss of tonic gravitational otolith stimulation. Adaptation to microgravity also alters neuromotor systems by modifying the repertoire of motor command strategies and synergies used for movement control in response to the sudden redistribution of forces along the body, reductions in the biomechanical support reactions, and alteration of relationships between motor command and body movement. These inflight sensory-motor adaptations disrupt postflight postural equilibrium control.

The primary goal of these investigations is to further expand our understanding of the central adaptive mechanisms responsible for the appearance and amelioration of postflight postural ataxia. Building on the substantial neuromotor information base that our IBMP group has amassed from primarily long

duration missions, and the similarly substantial neurosensory information base that our JSC group has amassed from primarily short duration missions, we have developed a joint protocol, employing key elements of both the standard IBMP test procedures and standard JSC test procedures, to which crew members were subjected before and after flight. Findings from these subjects will allow us to begin making direct comparisons of the independent techniques used in the two space programs, and should lead to new insights into how data from the Russian and U.S. information bases can be combined. By combining these two information bases, we will be able to make the first large n evaluations of the mechanisms of sensory-motor readaptation after space flight and the effects of mission duration on postflight postural ataxia.

The long term objective of this project is to determine the role of central adaptive mechanisms in reorganizing postural equilibrium control in humans subjected to long duration space flight. Ultimately, this knowledge will lead to development of effective countermeasures to the untoward effects of sensory-motor adaptation to space flight, and will improve our understanding of the adaptive processes required to compensate for clinical deficits in sensory-motor function.

Data Collection

- A total of 6 crew members from joint US/Russian missions participated in pre- and postflight data collection sessions: 3 from MIR-18, 1 from STS-71, and 2 from MIR-19.
- Loss and/or rescheduling of all the R+1, 2, 4, and 8 data collection sessions will severely limit our ability to compare these long duration results with our existing short duration data base.

Data Processing and Analysis

- Preliminary processing of the Paradigm 2 neurosensory control test data has been completed using off-the-shelf software. Comparisons of these long duration data with our existing short duration database has begun.
- A custom software system to perform detailed analysis of various kinematic and kinetic indices of postural sway has been completed in-house. Analysis of these parameters has begun for both Paradigm 1 neuromotor control test data and Paradigm 2 neurosensory control test data.
- A custom software system to analyze the EMG data is under development in-house. Processing of EMG data will begin after the software system is complete.
- All pre- and postflight data along with all of our completed data analysis software routines have been provided to our IBMP colleagues. They are responsible for analyzing the push stick data, as well as all of the Paradigm 1 neuromotor control EMG data. They are awaiting completion of a custom software package, currently being developed in their laboratory, before beginning their data processing and analysis.

Preliminary Science Findings

- Balance control deficits following long duration space flight appear to be far more profound than those following short duration space flight: two of the three Mir 18 subjects were too ataxic to attempt the balance control testing on R+0, while only four of 45 short duration crew members were that severely affected.
- The nature of postflight balance disturbances appears to be the same following long and short duration space flight missions: returning crew members are unable to adequately use vestibular system inputs.
- Neuro-motor disturbances persist for a much longer period following long duration flight than following short duration flight. Furthermore, they appear to be affected by changes in available sensory information.
- Complete recovery is substantially delayed following long-duration missions: the slow phase of the recovery process appears to progress much more slowly than with short-duration subjects.
- We intend to combine the results from the three Mir 18 subjects with those from two Mir 19 subjects before publication. We also intend to combine the results from the STS-71 subject with previous results from two STS-60 subjects to aid in comparisons between long and short duration missions.

This project seeks to improve our understanding of the mechanisms of basic adaptive responses of the brain to sudden, sustained changes in sensory input. While its primary focus is to examine the adaptation of the balance control system to loss and reintroduction of the tonic otolith stimulation provided by gravity, its results will also improve our understanding of the recovery processes of patients suffering from vestibular system loss or dysfunction. The experimental and analytical techniques developed for this project may also be useful for clinical assessment of balance disorders in the future.

Microbiology

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Funding:

Project Identification: 5.1

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 12/97

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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 crew members 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 and in the space station's microgravity closed system environment. Furthermore, the confinement of crew will allow for alterations in body microflora and transfer of microorganisms among crew members directly and through the environment. Air, water, interior surfaces and crew member 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, 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 crew members before, during, and after a 90 day mission to the Mir space station, (2) to determine extent of microbial transfer among crew members, 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.

The goal of this study was to characterize the microbial ecology of the crew members and the internal environment of the Mir space station during a 115-day mission. The Shuttle-Mir Program provided a unique opportunity to study the effect of long-duration space missions on the dynamics of crew and spacecraft microbiota. Crew member samples were collected before and after; air, water, and interior surface samples were collected during flight. Crew samples were analyzed with methods approved for Space Shuttle crew's medical evaluations. Surface, air, and water samples were processed and quantitated on orbit, then colonies were isolated and identified upon their return to the Johnson Space Center. Bacteria were identified by using the Vitek, BIOLOG, and MIDI identification systems, and fungi were identified by morphology and biochemical analysis.

Crew member clinical microbial findings were typical of healthy individuals and no changes were apparent in postflight microflora. Preliminary analysis is indicated transfer of human bacteria to the Mir surfaces, air, and water systems, as well as transfer of Staphylococcus aureus among crew members. The prelaunch human microbial bioload was typical of microbiota associated with different anatomical sites in healthy individuals. A slight increase however, was noted in postlaunch samples, after which the counts gradually declined and returned to prelaunch values 14 days after landing. Although no incidence of urinary tract infection was reported, the bacterial counts in urine were somewhat high. Types and numbers of microbes isolated from the Mir air and surfaces were little different from previous Space Shuttle flights. Representative microbes from air and surfaces included Aspergillus, Bacillus, Penicillium, and Staphylococcus spp. The microbial constituents of the Mir water system were quite different from that of the Space Shuttle. Bacillus and Staphylococcus spp. were isolated from the Mir recycled water while Bacillus, Methylobacterium, Rhodococcus, and Pseudomonas spp. were found in Mir ground-supplied water. Only Burkholderia cepacia was isolated from the Space Shuttle water system. The overall microbial quality of the Mir space station air, surfaces, and water equaled or exceeded that of the Space Shuttle.

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" (BRS). 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.

Publications, Presentations, and Other Accomplishments:

Koenig, D.W., Bell-Robinson, D.M., Johnson, S.M., Mishra, S.K., Sauer, R.L., and Pierson, D.L. "Microbial analysis of water in space." Proceedings of the 25th International Conference on Environmental Systems, San Diego, CA, 1995.

Viral Reactivation

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Project Identification: 2.4.3

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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 investigation was removed from the Mir 18 mission and remanifested for the Mir 19 mission; the planned inflight sample collection for the Mir 18 mission was suspended.

The objective of this experiment, to be conducted on Mir-19, 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.

One Mir-18 crew member collected five samples near the end of the mission. All samples were stored frozen and returned to the Johnson Space Center for analysis. The DNA was extracted from the preflight and limited inflight saliva specimens by the RNAzol method. An oligodeoxynucleotide primer set capable of detecting herpes simplex virus (HSV), types 1 and 2, cytomegalovirus (CMV),

and Epstein-Barr virus (EBV) was used for PCR analysis of each specimen. All preflight samples were negative for the presence of HSV, CMV, or EBV. PCR analysis demonstrated the presence of herpes virus DNA in two of the five inflight saliva samples. This study provided the first evidence of latent virus in crew member saliva during a space mission. Inflight saliva specimens from the Mir-19 crew members were delayed in their return to JSC, and they are currently being analyzed.

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 polymerase chain reaction (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.

Publications, Presentations, and Other Accomplishments:

Pierson, D.L. and Konstantinova, I. "Reactivation of latent virus infections of the Mir flight crew." Presented at the SMSP Phase 1A Workshop, USRA, Houston, TX.

Stowe, R. and Pierson, D.L. "A spreadsheet macro for setting up PCR assay tubes." Submitted to BioTechniques.

Patent Pending, U. S. Patent #: Undetermined Stowe, R.P. and Pierson, D.L. "A novel set of primers for detection of cytomegalovirus DNA in body fluids and tissue."

Patent Pending, U. S. Patent #: Undetermined Stowe, R.P., Mishra, S.K., and Pierson, D.L. "A novel polymerase chain reaction (PCR) method for rapid detection of herpes viruses."

Physiologic Alterations and Pharmacokinetic Changes during Spaceflight

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Funding:

Project Identification: 2.3.1

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Emergency and preventive medications are provided on all US space flights. Scientists believe that weightlessness affects the body's ability to use drugs effectively. The rate of a drug's absorption from the gastrointestinal (GI) tract and its rate of breakdown - primarily in the liver - are the main factors controlling the availability of the drug to the body, and therefore its effectiveness after administration. Because of this, it is important to understand any changes that occur in the rate of drug absorption, metabolism, and excretion (together know as pharmacokinetics) during weightlessness. This knowledge will be useful in the development and validation of new methods of drug treatment and delivery to assure effective drug therapy during space flight.

The objective of this experiment is to determine the changes in physiological and pharmacokinetic parameters during long-duration missions. Pharmacological tracers (e.g., acetaminophen and antipyrine) are used to determine the rate of absorption and elimination of drugs during long-duration missions. The experiment consists of two parts. The first part examines changes in GI motility during space flight. The protocol involves ingesting a special sugar and collecting breath samples to measure how the body metabolizes it. The second part involves measuring changes in drug metabolism in the liver by determining the level of metabolite in the urine. Both portions of the experiment are conducted during the Mir-18 mission.

Physiologic and Pharmacokinetic Changes During Space Flight

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Funding:

Project Identification:

Solicitation: 94 OLMSA-01

Initial Funding Date: 7/94

Expiration: 6/97

FY 1995 Funding: $56,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

The overall task was to identify physiological and pharmacokinetic changes in crew members during long-duration missions. The specific tasks were: 1) Identify changes in gastrointestinal motility during space flight using the lactulose-breath hydrogen test, 2) Determine gastric emptying time, absorption, bioavailability and elimination of acetaminophen during space flight, 3) Identify changes in hepatic metabolism activity during space flight by measuring the clearance of orally administered antipyrine, 4) Correlate physiological changes in GI and hepatic function with pharmacokinetic changes in acetaminophen absorption and metabolism.

The investigation consists of two interrelated protocols designed to evaluate changes in gastrointestinal and hepatic function induced by space flight. The first protocol involves assessing gastrointestinal function (an important contributor to pharmacokinetic variability during flight) using Lactulose Breath Hydrogen (LBH) test, a noninvasive and indirect method for measuring GI motility. Acetaminophen was also used to evaluate pharmacokinetics during flight. The second protocol involves assessing hepatic metabolism, an important determinant of drug and nutrient disposition. The metabolic activity of liver enzymes, expected to change during space flight, will be determined indirectly by measuring antipyrine clearance after oral administration.

Our task can be broken down into two components: A GI experiment and a Hepatic Experiment.

The GI experiment began with an 8-10 hour fast, after which crew members collected a saliva and a urine sample and two consecutive breath samples. Following a prescribed low fiber breakfast, crew

members ingested 650 mg of liquid acetaminophen, followed by orange juice containing 20 g of lactulose. After drinking the mixture, crew members collected serial breath samples, saliva samples and void-by-void urines as per the schedule outlined on the cue card. All inflight samples were identified and had collection times recorded with a bar code reader. Eighteen breath samples were collected in 6 hours, twelve saliva samples in 8 hours, and void-by-void urines for 24 hours post-ingestion of dose.

The hepatic experiment also began with an 8-10 hour fast, after which crew members collected a saliva and a urine sample. They ingested a 1.2 g antipyrine dose after consuming a prescribed low fiber breakfast. After the dose, crew members collected eight saliva samples and void-by-void urines for 48 hours post-ingestion of dose. All inflight samples were identified and had collection times recorded with a bar code reader.

Two crew members participated in the study. One preflight Baseline Data Collection (BDC) session for both the GI function study and Hepatic function study was completed before L-30 days. Three inflight sessions (on FD35 and 38, FD54, and FD88) were completed for the GI function study. Two inflight sessions for the Hepatic function study were completed on FD33 and FD99. One post flight session was completed for both the protocols between R+4 and R+7 days.

All biological samples were analyzed by appropriate methods to determine breath hydrogen methane levels and respective drug metabolite concentrations (184 breath samples, 180 saliva samples and 106 urine samples)

Preliminary Science Findings:

Gastrointestinal motility (GIM), a contributing factor of gastrointestinal function decreased 25-50% during flight. Breath methane and hydrogen levels were several fold higher during flight than on the ground. Mir air has correspondingly high methane levels. High methane may be a result of a combination of altered GI bacterial flora, GI stasis, and the methane rich environment of the Mir. Absorption of liquid dosage forms (Tylenol syrup) are less affected by GIM changes than solid dosage forms. Availability of acetaminophen was variable during flight and correlated well with GIM.

Hepatic function, a key factor for the metabolism of medications and toxicants, is governed by the hepatic enzyme activity and blood flow. Hepatic metabolism (HM) was variable during flight with a more than 50% decrease in one crew member and a 30% increase in the other. Postflight, HM was 20% less than that of preflight.

The preservative-coated salivettes and preservative discs for ambient storage of biological fluids (patent pending) have a wide range of application in clinical and research settings (remote site diagnostics clinics on the wheels, home health care, etc.). These tests can be used by clinicians for the noninvasive assessment of GI and hepatic function in gastric and pediatric diagnostics and treatment.

Publications, Presentations, and Other Accomplishments:

Putcha, Lakshmi. "A summary of preliminary observations." Phase 1A Data Sharing Workshop. NASA Johnson Space Center, Houston, TX, October 23, 1995.

Putcha, Lakshmi. "The data was presented at the poster presentation." Mr. Dan Goldin, NASA Administrator.

The Effects of Long Duration Space Flight on Gaze Control

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Funding:

Project Identification: 4.2.1

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

This proposal represents a joint U.S./Russian Sensorimotor investigation developed by using tasks that have been studied by U.S. investigators as a part of the Extended Duration Orbiter Medical Project, and similar tasks employed by Russian investigators on long duration MIR missions. These experiments (U.S. and Russian) have been combined to provide a common set of experiments designed to investigate the evolution (or emergence) of those goal-oriented strategies required to maintain effective gaze when the interactive sensorimotor systems required for gaze have been modified as a function of exposure to the stimulus rearrangement of space flight. We hypothesize in part: (1) that goal-oriented behavior in maintaining effective goal-oriented gaze will be modified by new strategies that maximize the positive aspects of visual dominance and the negative aspects of head movements during on-orbit performance and immediate postflight behavior; (2) that astronauts' spatially oriented perception and consequent compensatory action initially exhibits increased reliance on extrinsic spacecraft coordinates (perhaps driven by the initial reliance on vision), but that an intrinsic coordinate system becomes more heavily weighted as mission duration increases; (3) that in space flight with gravity removed from the equation, orientation vectors may be established with reference to intrinsic and extrinsic coordinate systems that determine response vectors (i.e., the direction of the eye velocity vector during flight attempts to align with intrinsic coordinates, and that the primary axis of orientation, unlike that observed when the stimulus is aligned with gravity, is the body Z axis), and that once a head movement has been initiated, immediate control of the head's position in space will be

compromised (due to space flight induced changes in proprioception), and that without appropriate feedback, target acquisition and other tasks requiring head control will be affected. It is our objective to use the following tasks to test the above hypotheses: (1) Target Acquisition:, (2) Pursuit Tracking, (3) Sinusoidal Head Oscillations (head shakes), (4) Memorized Head Rotations, and (5) Test For Both Spontaneous and Gaze Nystagmus.

A total of 7 astronaut and 4 cosmonaut subjects were obtained from the STS- 60, STS-63, STS-71/Mir 18 flights. Of these subjects the three from Mir 18 represent the first long duration sensory-motor data obtained in the U.S. Program since Skylab. Baseline data collection hardware and flight training hardware have been installed at Star City. We are currently finishing baseline data collection with the Mir 23 crew. Training for this flight was completed in January.

Equipment and software to accomplish all of the experiment functional objectives is now currently aboard the Mir Station.

A preliminary analysis of the data suggests that:

• Although there may be significant delays, subjects are able to acquire targets.

• Inflight and immediately postflight head velocity is reduced, and there appears to be a new neural strategy developed that trades the reduced head velocity (and in some cases smaller head displacements) with an increased VOR gain.

• Time to acquire known targets is increased. This represents a serious decrement in performance.

• Time inflight is positively correlated with increased error duration of effects. Very long duration flights, depending upon postflight levels of activity, may result in lasting effects that recover very slowly.

• Pursuit tracking is greatly impaired, and VOR suppression (consistent with an increase in gain), appears to be inhibited. This results in retinal errors beyond the acceptable limits.

The hardware required to support this experiment 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 modify completely 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 insure the safety, health and well being of future astronauts.

Publications, Presentations, and Other Accomplishments:

Glasauer, S., Bloomberg, J.J., Reschke, M.F., Peters, B.T., Smith, S.L., and Berthoz, A "Spatial orientation during locomotion following space flight." Man in Space Symposium, Toulouse, France, March, 1995.

Reschke, M.F., Bloomberg, J.J., Paloski, W.H., Harm, D.L., Parker, D.E. "Neurophysiologic aspects: Sensory and sensory-motor function. In: Nicogossian, A.E., Leach, C.L., Pool, S.L., eds. Space Physiology and Medicine." Lea & Febiger, Philadelphia , PA, pp 261-285, 1994b.

Reschke, M.F., Harm, D.L., Bloomberg, J.J., and Paloski, W.H. "Chapter 7: Neurosensory and Sensory-Motor Function. In: A.M. Genin and C.L. Huntoon, eds. Space Biology and Medicine, Vol. 3: Humans in Spaceflight, Book 1: Effects of Microgravity." AIAA, Washington, D.C., In press, 1995.

Reschke, M.F., Harm, D.L., Parker, D.E., Sandoz, G.R., Homick, J.L., Vanderploeg, J.M. "Neurophysiologic aspects: Space motion sickness. In: Nicogossian, A.E., Leach, C.L., Pool, C.L., eds. Space Physiology and Medicine." Lea & Febiger, Philadelphia, PA, pp 228-260, 1994a.

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Funding:

Project Identification: 7.1.2

Solicitation: AO-OSSA-84

Initial Funding Date: 10/93

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Students Funded Under Research: 10

Flight Information:

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

Responsible NASA Center: Ames Research Center

Task Description:

The microgravity environment of the Mir Space Station provides an outstanding opportunity for scientists to study the effects of gravity on plant life. In this experiment, investigators also hope to evaluate whether plants grown in microgravity can be used effectively in life support systems. The primary objective of the Greenhouse experiment is to perform a seed to seed experiment in space to determine the effect of microgravity on the productivity of crop plants; in this case, dwarf wheat. Additional objectives are to identify the chemical, biochemical, and structural changes in plant tissues induced by microgravity and to determine microgravity's effects on processes such as photosynthesis, respiration, transpiration, stomatal conductance, and water use. Another major objective is to evaluate the suitability of the facilities on Mir for advanced research on growing plants. The Greenhouse experiment is conducted in the Russian/Bulgarian developed plant growth facility, the Svet, to which the U.S. is adding an instrumentation system to gather information on how microgravity affects the gas exchange process and other environmental parameters. This instrumentation includes infrared (IR) gas analyzers to monitor CO_2 and water vapor as air enters and then leaves each of two plant cuvettes; that is, there are four IR analyzers. In addition, the U.S. instrumentation monitors O_2, air and leaf (IR) temperatures, cabin pressure, irradiance, and substrate moisture (16 probes in the root module). Facility modifications were performed during Mir 19, which began after STS-72 left Mir. Plant development was monitored by daily observations and photographs. Plant samples were collected for fixation or drying at five specific stages during development and at final harvesting. All samples were returned to Earth for post-flight analysis on STS-74.

During FY1995, our instrumentation was sent to Mir (on Spektr and on STS 72), and the equipment was added to Svet (the Russian/Bulgarian plant growth chamber sent to Mir in 1990) during late July and early August (Mir 19). Seeds were planted on August 13, 1995, and plants were sampled five times as planned plus the final harvest. Chemically fixed and dried samples plus the light bank and root module were returned on STS 74, November 20, 1995 (FY96; Mir 20). The experiment was a success in the sense that US equipment was tested and found to work well, but there were failures of Svet that led to very poor growth of the plants: Four of six small fluorescent lamps failed, three of these when the experiment was first turned on, and the controller failed, which meant that lights had to be turned on and off manually, as did the watering system. In addition, the MIPS failed so that it was not possible to have environmental data downlinked as planned. This made it extremely difficult to direct the cosmonauts when to add water, etc. Temperatures rose on three or four occasions because the fan that cooled the lamps failed to operate, and the substrate had an excess of water for much of the time, with a sharp gradient in moisture level from the point where water was added to the substrate just a few centimeters away. The plants growing under such low light, with an unplanned eight hour dark period (lights were supposed to be on all the time), and being exposed to nearly lethal high temperatures, barely stayed alive for the 90 days of the experiment. As far as we can tell, all plants were completely vegetative (i.e., no wheat heads). Heads normally form even under low light conditions, and wheat heads have previously formed in space. We are looking at various reasons why plants might have been vegetative, and we are preparing for a repeat of the experiment during FY96-97.

Plant physiologists have studied plant responses to gravity for well over a century, and we still have little understanding of how a plant can respond to even slight changes in the direction of gravitational acceleration. A vertical stem of a seedling, tipped less than 5o from the vertical, will reorient to the vertical within a few hours. The experiments in Mir should provide additional insights into this phenomenon. On Earth, cereals such as wheat often fall over (lodge) in the field. A better understanding of plant responses to gravity might lead to ways to increase a plant's ability to grow vertically again after lodging. In addition, the project has involved many ground based studies of wheat growth in response to various environmental parameters, and the results might find application in agriculture. It is hoped that we will be able to grow wheat as a food source for astronauts, purifying the atmosphere in the process.

Publications, Presentations, and Other Accomplishments:

Bingham, G.E., F.B. Salisbury, L.S. Gillespie and W. Goncalves (Abstract) "Diagnostic Equipment for the "Greenhouse 2" Experiment on the Russian Space Station Mir", Am. Soc. for Gravitational and Space Biology, Bulletin for the 10th Annual Meeting, San Francisco, CA, Oct 19-22, 1994, 84, 1994.

Bingham, G.E., F.B. Salisbury, W.F. Campbell, J.G. Carman, D.L. Bubenheim, B. Yendler, V.N. Sytchev, Y.A. Berkovitch, M.A. Leveiskikh and I.G. Podolsky "The Spacelab-Mir-1 "Greenhouse-2" experiment", Advances in Space Research, vol 18, No 4/5, 225-232, 1995.

Bingham, G.E., F.B. Salisbury, W.F. Campbell, J.G. Carman, D.L. Bubenheim, B. Yendler, V.N. Sytchev, Y.A. Berkovitch, M.A. Levinskikh and I.G. Podolsky "The Spacelab-Mir-1 "Greenhouse" experiment." (Abstract-p43) 30th COSPAR Scientific Assembly, Hamburg, Germany, July 11-21, 1994.

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Salisbury, F.B. NASA Tech. Memorandum, 1992-1993 NASA Space Biology Accomplishments (Contribution: Developmental studies of wheat in microgravity). NASA Tech Brief, 95-97, 1994.

Salisbury, F.B. "Suggestions for crops grown in controlled ecological life-support systems, based on attractive vegetarian diets." (abstract, p29) 30th COSPAR Scientific Assembly, Hamburg, Germany, 11-21 July, 1994.

Salisbury, F.B. "Controlled, Ecological, Life-Support Systems (CELSS): Some Historical Perspective." (Abstract, #539, p A53) Aerospace Medical Association, 66th Annual Scientific Meeting, Anaheim, CA, May 7-11, 1995.

Salisbury, F.B. (Abstract) "Some Experiences of Designing a Semi-Closed, Bioregenerative, Life-Support System for Use in Space Exploration: Implications for Terrestrial Ecology", Bulletin of the Ecological Society of America, 80th Annual ESA Meeting, Snowbird, Utah, 30 July-3 August, 1995, 235, 1995.

Salisbury, F.B. and M.A.Z. Clark "Suggestions for crops grown in controlled ecological life-support systems, based on attractive vegetarian diets", Advances in Space Research, vol 18, no 4/5, 33-39, 1995.

Salisbury, F.B., G.E. Bingham, L.S. Gillespie, J.G. Carman, W.F. Campbell and D.L. Bubenheim (Abstract) "Preparations for the "Greenhouse 2" Experiment on the Russian Space Station Mir", Amer. Soc. for Gravitational and Space Biology, Bulletin for 10th Annual Meeting, San Francisco, CA, Oct 19-22, 1994, 70, 1994.

Salisbury, F.B., G.E. Bingham, W.F. Campbell, J.G. Carman, D.L. Bubenheim, B. Yendler and G. Jahns "Growing super-dwarf wheat in Svet on MIR", Life Support and Biosphere Science, vol 2, no 1, 31-39, 1995.

Salisbury, F.B., G.E. Bingham, W.F. Campbell, J.G. Carman, P. Hole, L.S. Gillespie, V.N. Sychev, Y. Berkovotch, I.G. Podolsky and M. Levinskikh (Abstract) "Growing Super Dwarf Wheat on the Russian Space Station Mir", Amer. Soc. for Gravitational and Space Biology , Bulletin for the Eleventh Annual Meeting, Crystal City, VA, Octover 25-29, 1995, 63, 1995.

Salisbury, F.B., L. Gillespie and G. Bingham "Preparations for CELSS flight experiments with wheat", Advances in Space Research, vol 14, no 11, 21-27, 1994.

Salisbury, F.B., W.F. Campbell, J.G. Carman and G.E. Bingham "Growing Super-Dwarf Wheat Through a Life Cycle on Space Station Mir", (abstract, p 173) Life Sciences and Space Medicine Conference '95, Houston, TX, April 3-5, 1995.

Humoral Immunity

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Funding:

Project Identification: 2.4.2

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/ 94

Expiration: 9/ 95

FY 1995 Funding: $86,000

Students Funded Under Research: 0

Flight Information:

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 crew member 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.

The experiment was performed during the docked phase of the Mir 18/STS 71 mission. Additional sample collection occurred during the preflight and postflight periods. 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). Analysis of the salivary levels of the antigen specific antibodies is in progress. The samples are currently being analyzed for the levels of serum

immunoglobulins (IgG, IgA, IgD, IgE and IgM). Analysis of salivary IgA levels (antigen specific and total) and lysozyme levels will be performed in FY96.

The data from the Mir 18/STS 71 missions constitute the first part of this investigation. The data were intended to include additional subjects on Mir 19. However, the experiment was not performed during this flight. 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 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.

Peripheral Mononuclear Cells

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Funding:

Project Identification: 2.4.4

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 9/ 95

FY 1995 Funding: $88,000

Students Funded Under Research: 0

Flight Information:

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 micro-gravity 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 crew members. 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.

This experiment was performed during the docked phase of the Mir 18/STS-71 mission. Sample collection was performed during the preflight and postflight time frames in addition to the samples collected during flight. The analysis of the flow cytometry samples (preflight, inflight and postflight) has been completed. The assessment of natural killer cell phenotype and function is also complete. Peripheral mononuclear cells were activated to evaluate changes in lymphocyte function. The examination of early activation markers by flow cytometry has been performed on these samples. Cell supernatants and packed cell pellets were also collected from the activation studies. The supernatants

will be assayed for the levels of secreted cytokines. RNA will be isolated from the packed cell pellets and mRNA levels of cytokines will be measured. The cytokine studies (mRNA and secreted) are the final samples remaining to be processed (FY96).

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.

Publications, Presentations, and Other Accomplishments:

Patent In Process, U. S. Patent #: Undetermined, Sams, Clarence "Whole Blood Staining Device."

Evaluation of Skeletal Muscle Performance and Characteristics

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Funding:

Project Identification: 4.1.1

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $89,385

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Muscles that are not used lose their strength. In addition t 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. Reduced fitness occurs decreases in strength, endurance, tone, and efficiency. 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 (and the mechanisms 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 help evaluated 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.

- Mir 18/STS-71 before, during and after flight data was collected. Not all scheduled data takes were possible due to management and flight surgeon decisions.
- Data analysis is about 70% complete and has suggested a mechanism for the decrements in strength.

- The role of antagonist muscles during strength testing will be examined in the future.
- The interaction of reflexes (H-reflex, T-reflex, Functional Stretch Reflex) also may be playing a role in changes in muscle performance.
- Changes appear to have a larger neural vs morphological etiology.

The decrease in muscle tone, lower motorneuron pool sensitivity, and altered peripheral nerve proprioception may lead to an increase in co-contraction from antagonist muscles and a decrease in neuromuscular efficiency. This relationship may be mitigated with a variety of neuromuscular and exercise countermeasures in the future. Earth benefits lie in an increased understanding of muscle and muscle deconditioning. It is possible that some of the countermeasures used in space may be beneficial to physically handicapped subjects, such as people with cerebral palsy. These patients have similar functional changes that might benefit from space based research.

Maximal Aerobic Capacity Using Graded Bicycle Ergometry

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Funding:

Project Identification: 3.2.1

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $95,938

Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Mir-18/STS-71)

Responsible NASA Center: Johnson Space Center

Task Description:

Several exercise technique and devices have been used in both the American and Russian space programs. Exercise programs have been partially effective in maintaining a degree of physical conditioning, thereby reducing some of the deconditioning effects associated with space flight. Aerobic capacity is a good measure of exercise endurance and is used to prescribe good cardiovascular exercise. Direct assessment of aerobic capacity before, during (STS-71) and after long duration flight has not been done and will provide a measure of the efficacy of the Russian countermeasure program. Assessing cardiac output during the exercise may help define mechanisms that are associated with maintenance or loss of aerobic function.

Therefore, the purpose of this experiment was to quantitate the effectiveness of the Russian countermeasures and to identify key mechanisms that helped maintained or were responsible for decreases in aerobic capacity. Postflight responses were measured to determine the rate of readaptation. Aerobic capacity was determined on supine cycle and was graded from low to maximal levels of exertion. Aerobic capacity during upright exercise (treadmill) was shared from the SMSP #4.1.1 experiment.

- Mir 18/STS-71 before, during and after flight data was collected. Not all scheduled data takes were possible due to management and flight surgeon decisions.
- The small number of subjects preclude any general conclusions.

- Data analysis is complete and has suggested that changes in stroke volume is the key factor for changes in aerobic capacity.
- Regardless of test modality or position (upright vs supine), subjects who completed the most exercise had the smallest decreases. No subject was able to maintain their capacity.
- Exercise performed by the crew (not exactly as prescribed) was only partially effective. It is unknown if the Russian countermeasures (as prescribed) will work due to lack of compliance.

Comparison of the passive treadmill and cycle data showed that exercise efficiency decreased when levels of mechanical work are self selected (treadmill). The decrease was not due to metabolic measurements, since they were consistent, but were related to changes in biomechanics. This change probably had a neurological bases. This pilot work shows the need to perform multiple modality testing with astronauts and probably should be done with a variety of physically handicapped subjects. These results also showed the utility of operationally related testing (treadmill) versus cycle evaluations.

Renal Stone Risk Assessment

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Funding:

Project Identification: 2.1.3

Solicitation: US/RSA Negotiations

Initial Funding Date: 1994

Expiration: 1996

FY 1995 Funding: $80,000

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Data from previous missions suggest that space flight exposes crew members to a greater risk of forming kidney stones. Investigators believe that the risk increases with the duration of the mission. This investigation attempts to determine the degree of risk involved during extended space flight and to determine the factors which are affected by flight duration. Ultimately, medical investigators hope to use their understanding of increased in-flight kidney stone risk to determine ways to counteract the formation of these stones both in space and on Earth.

Three crew members from the Mir 18 mission have participated in this investigation. Urinary risk factor analysis is completed in the preflight, inflight, and postflight phases of this mission. Statistical analyses of these data have been completed and have been shared with the Russian investigator for review and discussions. Analyses of the dietary data investigating the contribution of environmental factors to renal stone formation is continuing.

Preliminary results have suggested the following conclusions: Increased calcium excretion and decreased urinary output as a result of exposure to microgravity altered the urinary chemical environment increasing the risk of calcium oxalate and calcium phosphate stone formation. In contrast to previous pre- and postflight renal stone risk analyses from Shuttle crew members, data from this study have demonstrated an increased risk for calcium phosphate stone formation during the inflight phase of the mission. Urinary output returned to preflight levels by R+6 but still remained very low. Calcium phosphate values returned to preflight levels at R+10 but the risk of calcium oxalate stone formation remained in the high risk range throughout the postflight period.

The data from Mir 18 are the first part of this investigation. The data obtained will be combined with the data from the Mir 21 and Mir 23 crews.

Approximately 12 percent of the Earth-bound population will develop a renal stone sometime during their lives. Initially, lessons learned from studies on Earth will be used to minimize the potential for renal stone formation in crew members 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 crew members.

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. 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. As an example, currently under development is a method of urine collection in which the urine is dried on a filter card, uses no preservatives, and can be stored at ambient temperatures for extended periods of time.

Measurements of Cytogenetic Effects of Space Radiation

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Funding:

Project Identification: 5.2.6

Solicitation: US/RSA Negotiations

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $147,500

Students Funded Under Research: 2

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

Space radiation consists of a wide range of energetic, charged particles. The capacity of these particles to cause mutagenic and carcinogenic effects can vary greatly depending on the charge and energy of the particle. Direct measurements of the biological effects of space radiation in the human body, particularly damage to DNA, are essential for the assessment of risk to crew members.

Investigators for this experiment have proposed that space radiation can cause changes of the genetic material of the body. The amount of damage depends upon the type of particles and the amount of exposure. The basic goal of this experiment is to use advanced molecular cytogenetic techniques to determine the extent of radiation-induced chromosomal damage in human white blood cells under microgravity conditions. Blood collected pre- and post flight is analyzed using state-of-the-art chromosomal painting techniques and a semi-automated image analysis system. The investigators use the radiation data collected by the "In-flight Radiation Measurements" investigation to determine crew doses. Correlation of crew doses and measured chromosomal damage will determine the biological effects of space radiation.

The analysis of L-14, R+0 and R+9 samples has been completed. Samples collected after R+9 are yet to be scored and analyzed. Preflight (L-14) samples were irradiated with gamma rays, and a dose-response curve for induction of chromosomal aberrations, including translocations, was generated. Postflight samples (R+0, R+9) were analyzed and dose absorbed in space was extrapolated from the

preflight calibration curves. Relative Biological Effectiveness (RBE) was calculated by comparing the dose so determined and the dose measured by physical dosimeters. Samples of L-14, R+0 and R+9 have been analyzed. The frequency of chromosomal aberrations for L-14, R+0, and R+9 sample was $2.8+0.99x10^{-3}$, $8.1+1.33x10^{-3}$, and $7.02+1.6x10^{-3}$ respectively. Clearly there was a significant increase of chromosomal aberration in postflight samples.

The data for reciprocal translocation frequency of L-14 samples exposed to various doses of gamma rays can be presented by a least square fitting equation: $Y=(2x10^{-3}) + (7.6x10^{-5})D + (2x10^{-6})D^2$, where Y is reciprocal translocation frequency, and D is the dose in cSv or rem. Based on this dose-response curve and the reciprocal translocation frequency found in R+0 and R+9 samples, the absorbed dose received by crews during the mission was equivalent to about 14.5 cSv or rem. Data for dicentrics gave similar results. The average RBE is about 3.5, since the absorbed dose measured by physical dosimeters is 4.16 cGy or rad for the entire mission.

The number of sister chromatide exchanges (SCE) in each lymphocyte was carefully scored for L-14, R+0 and R+9 samples, and no significant difference was found. The average frequency of SCE is about 4.3+0.3 per cell. These results indicate that chromosomal aberrations observed in this study are primarily induced by space radiation, not by chemical mutagens.

Experimental results clearly indicate that high-linear-energy-transfer charged particles, such as alpha particles of radon gas, can be very effective in causing genetic damages in human cells. Health risk of radon gas has been a major concern at certain places in this country. These data are relevant to radiation risk assessment for high altitude commercial flights.

For present study both chromosome painting or fluorescence in situ hybridization (FISH) and BrdU incorporation techniques were used. It is clear from this study that a combination of both techniques is effective and necessary for biodosimetry. Biodosimetry can be very important for determining health risk to workers who accidentally exposed to radiation.

Publications, Presentations, and Other Accomplishments:

George, K. and Yang, T.C. "Chromosomal translocations in human cells exposed to gamma rays." Proceedings of the 43rd Annual Meeting of Radiation Research Society, San Jose, CA, April 1-6, 1995. Abstract.

Wu, H., Goodwin, E. H., and Yang, T.C. "Spatial consideration in the formation of radiation-induced chromosome aberrations and the test of interaction distance hypothesis." Proceedings of the 43rd Annual Meeting of Radiation Research Society, San Jose, CA, April 1-6, 1995. Abstract.

Yang, T.C., George, K.A., Kavakoli, A., Craise, L.M., and Durante, M. "Radiogenic transformation of human mammary epithelial cells in vitro." Proceedings of the Workshop on Neoplastic Transformation in Human Cell Systems in Culture: Mechanisms of Carcinogenesis, Chicago, IL, September 7-9, 1995.

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Yang, T.C., George, K., and Tavakoli, A. "Radiation-transformed human mammary epithelial cells: Chromosome and cancer gene studies." Proceedings of the 43rd Annual Meeting of Radiation Research Society, San Jose, CA, April 1-6, 1995. Abstract.

Studies of Mechanisms Underlying Orthostatic Intolerance Using Ambulatory Monitoring, Baroflex Testing and the Valsalva Maneuver

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Funding:

Project Identification: 3.1.2

Solicitation: US/RSA Negotiations

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: Johnson Space Center

Task Description:

This investigation studies mechanisms of orthostatic intolerance using a baroreflex response measurement system. Arterial baroreceptors, located in the aorta and in the carotid arteries of the neck, constantly monitor blood pressure. When these receptors sense increasing arterial pressure, they respond by sending messages to the brain, producing a reflex decrease in heart rate. When the receptors sense decreasing arterial pressure, there is a reflex increase in heart rate.

The baroreflex response measurement device mimics increasing and decreasing arterial pressure by applying suction and pressure to the neck. The baroreceptors in the carotid arteries respond as if pressure were actually increasing and decreasing, and the device measures the resulting heart rate changes. This equipment also measures heart rate and blood pressure during rest and the Valsalva maneuver. Similar to purposely "popping" the ear drums when ascending or descending in an airplane, this maneuver involves straining against a closed glottis. This action changes pressures in the chest and restricts the return of blood to the heart.

Previous data has shown that heart rate responses to the same neck pressure/suction stimulus are reduced during and after space flights of 10 to 14 days. It is believed this decreased response may be a contributing factor to orthostatic intolerance. This experiment extends these findings by determining the extent to which these responses have deteriorated during the 90-day flight and number of days they remain depressed after landing. In addition, experimenters will attempt to determine what causes this

abnormality by relating experiment-induced responses to spontaneous arterial pressure and heart rate patterns occurring for the 24 hours preceding the test.

Analyses have been performed on all 24 hour Holter and carotid baroreflex data. Valsalva maneuver and stand test data are presently being reduced, but analyses have not been performed. Blood volumes, plasma catecholamine levels, and plasma renin activity levels have been received, but not evaluated.

This investigation of the carotid baroreceptor-cardiac reflex responses and cardiac dysrhythmias yielded important information about cardiac function during long-duration space flight. First, baroreflex attenuation near the end of long-duration (115 days) and short-duration (10 days) space flight is similar, but postflight recovery is delayed after long-duration space flight. Second, heart rate and atrial rhythm disturbances decline early in flight, but gradually increase with duration of space flight. Early declines are similar to short-duration space flight. Third, incidence of ventricular dysrhythmias were quite variable but generally remained elevated. This is different from short-duration space flight. Fourth, significant alterations in rhythm such as atrial and ventricular tachycardia were observed during and after long-duration space flight. None were reported during or after short-duration space flight.

Our findings in this and previous investigations of reduced carotid baroreflex function, heart rate, and incidence of atrial dysrhythmias suggest that significant cardiac adaptation occurs within the first days of space flight, probably the result of decreased sympathoexcitation. Unfortunately, as the space flight progresses, apparent alterations in the electrical conduction system of the heart leave the heart prone to potentially malignant alterations in rhythm. A myriad of causative factors may be responsible for this potentially serious impact to crew health and safety.

This research may help us to understand some of the basic biological processes involved in the regulation of the cardiovascular system. While this research is not directly targeted at a disease or malady that affects humans on Earth, its results may further the understanding of conditions which interfere with normal cardiovascular regulation. These include, but are not limited to, idiopathic hypotension, adrenergic failure, Shy-Drager syndrome, diabetes, spinal cord injury, and heart failure.

Protein Metabolism During Space Flight (SLS-1 and SLS-2)

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No Co-I's Assigned to this Task

Funding:

Project Identification: E120

Solicitation: 78 AO

Initial Funding Date: 1/95

Expiration: 12/95

FY 1995 Funding: $126,250

Students Funded Under Research: 3

Flight Information:

Flight Assignment: None

Responsible NASA Center: Johnson Space Center

Task Description:

The tasks for the final year of this project were to complete the analytical work and write up the papers describing the results. These goals have been accomplished and two papers were submitted (and accepted) for publication by the Journal of Applied Physiology. This project has now been completed.

We have now completed all work on this contract. During FY 1995, we completed the analyses and the preparation of various manuscripts and reports. The project has resulted in eight papers.

The early phases of human space flight are associated with a stress response with an increase in protein turnover, acute phase protein synthesis and pro-inflammatory cytokine activity. With increasing duration of flight, there was a trend for the whole body protein synthesis to be less than preflight.

Nitrogen retention was decreased during flight with the magnitude of the decrease lessening towards the end of the mission. There was a sharp drop in N retention for the first 1-2 days of flight followed by a 3-5 day catch up period. Afterward, N retention was less than preflight but decreased with increasing time in space.

Energy intake is less during flight than preflight. Preflight the mean energy intake was 39.0 ± 2.5 (10) kcal/kg/d. There was a sharp drop in dietary intake on flight day 1 with recovery by the second day and then energy intake was constant at 30.4 ± 1.5 (12) kcal/kg/d for the remainder of the flight period ($p<0.05$).

Even though the inflight energy intake was greater on Skylab (36.8 ± 1.3 (9) kcal. kg-1.d-1) than on the Shuttle (30.1 ± 1.5 (12) kcal. kg-1.d-1, $p<0.01$) Nitrogen retention was greater on the Shuttle

(16.3 ± 3.4 (11) mg N.kg-1.d-1) than Skylab (-18.5 ± 5.9 (9) mg N.kg-1.d-1, $p<0.01$). One obvious difference between the two missions was the degree of exercise. On Skylab, but not Shuttle, there was a specific prescribed exercise program which increased in intensity with each succeeding mission. The observations suggest that (i) food requirements for space missions will vary with the amount and type of work/exercise done, and (ii) an intensive inflight exercise program may be counter-productive for attenuating the space flight induced protein loss unless the associated increased energy needs can be met.

This project demonstrates the remarkable ability of humans to adapt in the short term to a totally novel environment to which there can be no specific preprogrammed genetic response. No extrapolations or inferences though should be made about long flights from this short term data.

Publications, Presentations, and Other Accomplishments:

Crispi, ML, DM Porterfield and ME Musgrave "Control of growth and reproductive development in *Arabidopsis thaliana* by non-earth normal metabolic gas ratios." ASGSB Bull., 9, 50 (1995).

Kuang, A, ME Musgrave and SW Matthews "Seed production under space flight conditions." Plant Physiol. (Suppl.), 108, 57 (1995).

Kuang, A, ME Musgrave, SW Matthews, DB Cummins and SC Tucker "Pollen and ovule development in *Arabidopsis thaliana* under spacef light conditions." Amer. J. Bot. , 82, 585-595 (1995).

Kuang, A, Y Xiao and ME Musgrave "Seed development in *Arabidopsis* under space flight conditions on STS-68." ASGSB Bull., 9, 54 (1995).

Musgrave, ME, A. Kuang, SW Matthews and KM Ramonell "Plant reproduction under space flight conditions." ASGSB Bull., 9, 92 (1995).

Porterfield, DM, ME Musgrave and T Dreschel "Rootzone morphology and alcohol dehydrogenase activity of dwarf wheat grown on nutrient delivery systems designed for microgravity application." Plant Physiol. (Suppl.), 108, 148 (1995).

Porterfield, DM, SW Matthews and ME Musgrave "Transcription, activity, and localization of alcohol dehydrogenase in the roots of *Arabidopsis thaliana* following exposure to space flight conditions." ASGSB Bull., 9, 16 (1995).

Stein, T.P. "Protein requirements for long term missions." Adv. Space Res., 14 (11), 157-166 (1994).

Stein, T.P. and Gaprindachvili, T. "Space flight and protein metabolism with special reference to humans." Am. J. Clin. Nutr., 60, 806S-819S (1994).

Stein T.P. and Schluter, M.D. "Excretion of IL-6 by astronauts during space flight." Am. J. Physiol., 266, E448-E454 (1994).

Stein, T.P., Leskiw, M.J., and Schluter, M.D. "Diet and Nitrogen metabolism during space flight in the Shuttle." J. Appl. Physiol., (In press).

Stein, T.P., Leskiw, M.J., and Schluter, M.D. "Addendum: Diet and Nitrogen metabolism during space flight in the Shuttle. Comparison of Shuttle against Skylab." J. Appl. Physiol., (In press).

Stein, T.P., Schluter, M.D., and Boden, G. "Development of insulin resistance by astronauts during space flight." Aviat. Space Environ. Med., 65, 1091-1096 (1994).

Pulmonary Function During Extended Exposure to Weightlessness

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Funding:

Project Identification: None

Solicitation: 94 OLMSA-01

Initial Funding Date: 1/95

Expiration: 2/95

FY 1995 Funding: $5,835

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Not Manifested

Responsible NASA Center: Johnson Space Center

Task Description:

The lung is extremely sensitive to gravity, and experiments we performed on Spacelabs SLS-1, SLS-2 and D-2 showed marked changes in pulmonary function in microgravity. We propose to use 2 US/Mir flights of 180 days duration to study the effects of long term exposure to microgravity on the lung. We will study the distribution of ventilation and perfusion, gas exchange, cardiac performance, lung volume changes, and heart rate using a specially designed Pulmonary Function Facility already planned to be on board Mir/Spektr. We expect to see changes in many of the parameters we measure as a result of long duration microgravity, and also due to continued exposure to CO_2 and closed environment habitation. The results from this study will shed light on the physiological consequences of extended space flight and on necessary provisions for continued long duration space flight.

An Experiment Document was produced. No funding beyond that has been allocated, and we are not manifested. No further development foreseen.

Spaceflight Effects of Mammalian Development

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Funding:

Project Identification:

Solicitation: 93 OLMSA-03

Initial Funding Date: 6/94

Expiration: 5/96

FY 1995 Funding: $298,073

Students Funded Under Research: 11

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

Responsible NASA Center: Ames Research Center

Task Description:

Dr. Alberts will study the fetal and postnatal development of rats to verify the hypothesis that microgravity reduces stimulation of the developing fetal vestibular system and alters early function. His studies will also emphasize the behavior and physiology that are known to contribute to successful pregnancy, labor, delivery, and the onset of postnatal care, especially lactation.

Details: The data expected can contribute to our understanding of basic vestibular function. This function has an important role in numerous disorders of movement and coordination, rehabilitation processes after injury and deterioration during aging.

Our progress thus far has been encouraging; many of the observations and results are new and exciting. We have completed the arduous task of encoding the behavior of pregnant rats within their observation cages. These animals were observed remotely under continuous time-lapse video surveillance (24 hr/day) , beginning soon after the recovery from space flight until they delivered their litters. Much was learned from these observations.

First, we can now provide quantitative data showing that the labor and vaginal deliveries of Flight rats and ground Control rats share many important parameters. Labors began at the same time. Labors lasted the same amount of time, the birth process was accomplished in the same duration, even the pup-to-pup intervals were equivalent. These findings combine nicely with equivalence in number and size of the offspring. Nevertheless, in contrast to this picture of equivalence in the birth process, we found that Flight mothers displayed *twice* as many labor contractions as did the Synchronous Control group.

Thus, we have been able to establish, contrary to the expectations of many researchers, that rat dams can sustain successful vaginal deliveries after spending half of their pregnancy in microgravity. The increased numbers of contractions suggests that significant muscle deconditioning may have occurred, and affected the force or duration of the contractions. We cannot answer such questions from the present data. But we were prepared by these results to focus on correlated histological measures of smooth and striated muscle in the subsequent, R2 mission.

Much of our work was designed to examine the sensory capabilities of the offspring. The major class of question being examined in our studies concerned the patency and functionality of the vestibular system in infant rats that were gestated in microgravity. We applied a battery of tests, each intended to emphasize different, but related aspects of vestibular function in the offspring. Such studies are fundamental and novel. They are predicated on the expectation that early stimulation of a sensory system contributes to the development of that system, and thus affects its function. In the visual system, for example, it is now well-established that patterned light is needed to stimulate the immature, developing visual system and, without such stimulation, the eye and the brain may not develop into a normal, fully functioning system.

One of the great promises of the NIH.R1 mission was to make an initial analysis of early vestibular function in rats that had altered (presumably reduced) vestibular function during gestation. Our efforts were rewarded.

The ability of an infant to "right" itself when placed on its back on a solid surface, i.e., turn from supine to prone, was no different in Flight and Control pups. This establishes the pup's basic competence, particularly in making the requisite movements for righting. When we eliminated the tactile and proprioceptive cues to the pup's back, by "dropping" the pup in warm water and observing its response, we saw poor vestibular-based responses in the Flight animals. In contrast, Control pups in the water-drop test showed rapid and efficient righting. The differences were no longer apparent by Day 5, when all pups showed similar responses.

In another vestibular test involving 30 sec of rotation, during which we normally observe a counter-rotational head deflection, followed by a post-rotary compensatory head deflection, Flight pups lacked the post-rotary response. Again, this flight-associated effect disappeared within a few days.

Both of these findings, i.e., the disrupted behavior of 2-day-old and 3-day-olds in the water-drop and rotation tests, suggest that Flight pups sustained an alteration in their vestibular responsivity, probably involving cues from the labyrinths. These findings have alerted us to scrutinize anatomical data expected from the R2 mission, where some vestibular neuroanatomy will be analyzed.

Our heart rate studies did not yield interpretable inter-group differences, but we did make some unanticipated observations of differences in heart rate variance between groups, suggesting possible cardiovascular changes in the Flight animals. We continue to study these datasets. Progress this year has directed our attention back to the in-flight data, where we can now search for clues that might help provide an interpretive context for the dramatic differences in pup behavior that we observed.

The research conducted as part of NIH.R1 was primarily and foremost a series of investigations into basic biological processes. As such, we have been treated to new knowledge. Some findings were quite unexpected and other portions were important as a validation of what "ought" to happen, but had never been tested. The basic 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 a microgravity, i.e., in the absence of normal vestibular 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 studies. In particular, we see these experiments as foundation studies for an understanding of how the vestibular and proprioceptive systems are established in the mammalian body, 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 the birth process 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 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 space station.

Publications, Presentations, and Other Accomplishments:

Alberts, J.R. "Gravid sans gravity." Winter Animal Behavior Conference, Jackson Hole, WY, January 1995.

Alberts, J.R. "Exploring Mammalian Development in Space." American Institute of Astronautics and Aeronautics, Houston TX, April 1995.

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S.Farrell, W.J. & Galvani, C.D "Maternal behavior and offspring development of NIH.R1 rats." American Society for Gravitational and Space Biology, Crystal City VA, October 1995.

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S. Farrell, W.J. & Galvani, C.D. "Video images of vestibular tests of postnatal rats gestated during spaceflight." American Society for Gravitational and Space Biology, Crystal City VA, October 1995.

Alberts, J.R. Ronca, A.E. , Abel, R.A., & Farrell, W.J. "Vestibular tests of postnatal rats gestated during spaceflight." International Society for Developmental Psychobiology, San Diego CA, November 1995.

Ronca, A. E. "Pregnant rats aboard the Space Shuttle." American Association for Laboratory Animal Science, Indianapolis IN, May 1995.

Ronca, A.E., Alberts, J.R., Abel, R.A., Armbruster, M.E., Cabell, K.S.Farrell, W.J. & Galvani, C.D. "Spaceflight effects on rodent pregnancy and parturition." American Society for Gravitational and Space Biology, Crystal City VA, October 1995.

Ronca, A.E., Alberts, J.R. Abel, R.A., & Farrell, W.J. "Pregnancy and parturition of rat dams onboard the Space Shuttle." International Society for Developmental Psychobiology, San Diego CA, November 1995.

Phantom Torso

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-07

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: STS-85

Responsible NASA Center: Johnson Space Center

Task Description:

No additional data was provided by the investigator for this research.

Adaptive Response of Slow and Fast Skeletal Muscle in the Monkey to Spaceflight

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 10/94

FY 1995 Funding: $143,586

Joint Participation: NIH

Solicitation:

Expiration: 10/96

Students Funded Under Research: 4

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

Responsible NASA Center: Ames Research Center

Flight Hardware Required: AEM

Task Description:

Dr. Bodine-Fowler is studying the effects of reduced gravity during embryogenesis, including the postnatal development of the neuromuscular system. She is also investigating whether the embryonic system requires gravity to establish proper innervation of muscles by spinal motor neurons, normal morphological development, and normal differentiation of muscle fibers and tendons. Finally, she will determine the time course and quality of adaptation of the neuromuscular system to terrestrial gravity after development in microgravity.

Details:
• These experiments will provide valuable information on how muscles develop, which could lead to advances in treatment of muscle diseases.

STS-70 launched and landed successfully in July 1995 and was 9 days in duration. Tissue was collected from Gl9, PN1, PN3, PN5, PN8, PN10, PN17, PN21 and PN35 animals, as well as, the hindlimbs of the Dams.

Analysis of the Sol and MG muscles from the Dams has shown that there was atrophy in some fibers of the Sol and MG. The atrophy in the MG appears to be restricted to the deep compartment of the muscle. There was an upregulation of fast myosin heavy chains (MHC) in the Sol. We are continuing the analyses of these muscles by examining changes in the transcription of the MHC mRNA isoforms.

We have just begun the analysis of the hindlimbs from the fetal and postnatal animals. We are examining the expression of the following proteins using immunohistochemistry: myosin heavy chains (embryonic, neonatal, slow, IIa, IIx, IIb), myogenin, myoD, SR fast. We are examining the expression of the following mRNA using *in situ* hybridization and RNase Protection Assay: myosin heavy chains (embryonic, neonatal, slow, IIa, IIx, IIb), myogenin, and myoD.

The data collected from this study will aid in understanding the role of gravity during the embryonic development of the neuromuscular system. One question that remains to be answered is whether normal development can occur in a weightless environment. If exposure to microgravity retards the development of the neuromuscular system we will be able to determine whether exposure to a normal gravity environment postnatally enables the system to recover to a normal state. The data collected in this study will provide basic information regarding muscle development and growth processes. This information may be useful in understanding diseases which cause atrophy and degeneration of muscle tissues.

Publications, Presentations, and Other Accomplishments:

Bodine-Fowler, S.C., Allsing, S. and Botte, M.J. Time course of muscle atrophy and recovery following a phenol-induced nerve block. Muscle and Nerve, (in press), (1995).

Bodine-Fowler, S.C. and Pierotti, D.J. Expression of 2x myosin heavy chain mRNA in the rat soleus following reinnervation. Neuroscience Letters, (submitted 1995).

Bodine-Fowler, S.C., Meyer, R.S., and Pierotti, D.J. Adaptation of the rat soleus to foreign and correct innervation following sciatic nerve injury. J. Appl. Physiol, (submitted, 1995).

Bodine-Fowler, S.C., Meyer, R.S., Moskovitz, A., Abrams, R.A. and Botte, M.J. Inaccurate projection of rat soleus motoneruons: A comparison of nerve repair techniques. Muscle & Nerve, (submitted 1995).

Bodine-Fowler, S.C., Pierotti, D.J. and Talmadge, R.J. Functional and cellular adaptation to weightlessness in primates. J. Gravitational Physiol., (in press).

Edgerton, V.R., Bodine-Fowler, S., Roy, R.R., Ishihara, A., and Hodgson, J.A. "Handbook of Phsiololgy: Integration of Motor, Circulatory and Metabolic Control During Exercise. Section A: Neural Control of Movement." (in press).

Huey, K.A. and Bodine-Fowler, S.C. Altered myosin mRNA and protein content in rat soleus and tibialis anterior following reinnervation. Am. J. Physiol., (submitted, 1995).

Merati, A.L., Bodine-Fowler, S.C., Bennet, T. and Ryan, A.R. Identification of a novel myosin heavy chain gene expressed in the rat larynx. Biochimica et. Biophysica Acta, (in press).

Meyer, R.D., Abrams, R.A., Botte, M.J., Davey, J.P. and Bodine-Fowler, S.C. Improvement of muscle force following crush and transection injuries to the rat sciatic nerve. J. Ortho. Res., submitted, (1995).

Saljooque, F., Huey, K.A., and Bodine-Fowler, S.C. Use of the Ribonuclease Protection Assay to Detect Myosin Heavy Chain mRNA Isoforms in skeletal muscles. J. Appl. Physiol., submitted, (1995).

Talmadge, R.J., Roy, R.R., Bodine-Fowler, S.C., Pierotti, D.J. and Edgerton, V.R. Adaptations in myosin heavy chain profile in chronically unloaded muscles. Basic and Applied Myology, 5, 114-134 (1995).

Unguez, G.A., Roy, R.R., Bodine-Fowler, S.C. and Edgerton, V.R. Limited fiber type grouping in self-reinnervated cat tibialis anterior muscles. Muscle Nerve, submitted, (1995).

Unquez, G.A., Roy, R.R., Pierotti, D.J., Bodine-Fowler, S.C. and Edgerton, V.R. Further evidence of incomplete neural control of muscle properties in cat tibialis anterior motor units. Am. J. Physiol., 268, C527-C534 (1995).

Investigations of the Effects of Microgravity on In Vitro *Cartilage Calcification*

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Funding:

Project Identification:

Initial Funding Date: 2/94

FY 1995 Funding: $115,124

Joint Participation: NIH

Solicitation: 93 OLMSA-04

Expiration: 1/96

Students Funded Under Research: 2

Flight Information:

Flight Assignment: NIH-C5 (STS-72, 1995)

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 proliferation, 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.

Analysis of the results from the C-2 experiment revealed that the land-based controls started to mineralize just before recovery, while the flight experiment showed no evidence of mineralization. The flight cultures showed evidence of excessive cell proliferation with little evidence of maturation.

Specifically, while ground controls formed abundant cartilage nodules, the flight cultures formed very few nodules.

To verify these findings, and test the initial hypothesis concerning mineral deposition in hypogravity, the study was repeated on the 1/14/96 STS-72 (NIH C5) flight. In these studies, the cultures were launched at a later time point to insure mineralization would occur. Unfortunately, the computer failure on the flight module prevented the cells from receiving gasses and media. The ground control is being analyzed, but no flight data could be obtained.

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 understand 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.

Publications, Presentations, and Other Accomplishments:

Boskey, A.L. "The effect of Spaceflight on Bone and Cartilage Cell Differentiation." Fifth International Conference on the Chemistry and Biology of Mineralized Tissues, Kohler Wisconsin, October 22 - 27, 1995.

Stability and Precision of Human Performance during a Spacelab Mission

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Funding:

Project Identification: E910
Solicitation: 89-OSSA-13 (IML-2)

Initial Funding Date: 5/95
Expiration: 8/97

FY 1995 Funding: $29,527
Students Funded Under Research: 1

Flight Information:

Flight Assignment: HP-1 (STS-80, 1996)

Responsible NASA Center: Johnson Space Center

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; to determine the relationship between subjective responses and cognitive and psychomotor performance.

Progress over the initial months of this funding period has focused upon equipment acquisition (Macintosh Powerbook 170), software development, and testing of programmed performance tasks.

The research undertaken on this task will help to 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.

Publications, Presentations, and Other Accomplishments:

Kelly, T., Emurian, C.S., Baseheart, B.J., Martin, C.A., and Hays, L.R. "Cumulative effects of alcohol on human behavior. In: Problems of Drug Dependence." Edited by: Harris, L.S. NIDA Research Monograph, In Press, 1995.

Starch Metabolism in Space-Grown Soybean Seedlings

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Funding:

Project Identification:

Solicitation: 93 OLMSA-05

Initial Funding Date: 7/95

Expiration: 8/95

FY 1995 Funding: $39,963

Students Funded Under Research: 3

Flight Information:

Flight Assignment: BRIC-03 (STS-63, 2/95)

Responsible NASA Center: Kennedy Space Center

Task Description:

This experiment tested the hypothesis that starch concentration in plant tissue is decreased due to the effects of the space/microgravity environment and investigated possible mechanistic causes for the changes in starch concentration. Measurements were made of starch and soluble sugar concentrations, critical biosynthetic and degradative enzyme activities, localization of the starch grains and the plastids in which they are found, structural and ultra structural make-up of different tissues within the plants, and detailed measurements of growth and biomass partitioning.

During this fiscal year two flights of the payload were successfully completed. The BRIC-01 experiment flew on STS-68 in September 1994. This was an 11-day mission on which two canisters filled with soybeans were flown. The soybeans were harvested post flight. The BRIC-03 experiment flew on STS-63 in February 1995. This was an 8-day mission. Two canisters of soybeans were flown. One was frozen in space after 5 days and the other returned unfrozen for post flight harvesting. Analyses for these experiments were completed. These included measurements of growth, gas concentrations in the canisters, carbohydrate concentrations and related enzyme activity measurements in the cotyledons and ultrastructural analysis of cotyledon, hypocotyl and root tissue sections.

The hypothesis that starch would be reduced in concentration in the space-grown cotyledons was supported by the results of these experiments. At each time point (5, 8, and 11 days) starch concentration in the cotyledons was reduced by approximately 25% in the space tissue compared to the ground controls. An ancillary clinostat experiment showed similar results, i.e. that the clinostatted soybeans had reduced starch in the cotyledons compared to the upright, stationary control plants. Measurements of 11 different enzyme activities related to starch and sugar metabolism were conducted. Only ADP glucose pyrophosphorylase, a rate-limiting enzyme in starch synthesis, was affected by the space flight environment. The activity of this enzyme was lower in the space-grown cotyledons

compared to the ground controls, suggesting that the lower starch concentration seen was due to a lower activity of this enzyme. Starch grain size in the cotyledons was also measured. It was found that starch grains were larger or not affected by the space flight environment. Taken together with the lower concentration of starch in the space-exposed cotyledons, this suggests that the starch grain itself may have an altered (i.e., less dense) structure in space. Finally, it was found that there was increased root growth in some of the space grown plants. This alteration in biomass partitioning may have been related to the higher ethylene concentrations in the space canisters compared to the ground controls.

In the future, work will continue in trying to elucidate the mechanism for the reduced starch concentrations in space-grown plant tissue. This will focus on studies of the regulation of the rate limiting enzyme ADP glucose pyrophosphorylase, which is lower in the space-grown tissue. We will investigate the structural properties of starch grains formed in space tissue in soybean and other plants (including potato tubers). We will conduct experiments to understand the interaction of ethylene and gravity in controlling the partitioning of biomass within plant tissue. These studies will be conducted as part of the Collaborative Ukrainian Experiment (Fall 1997) and other flight opportunities.

Results from the BRIC-01 and BRIC-03 experiments will lead to a more complete understanding of primary plant metabolism, particularly in the area of starch metabolism. As the human race ventures further (therefore longer) into space, it is critical that we understand how the space flight environment affects basic physiology and metabolism of all organisms. Long-term space flight missions will require long-term life support capabilities. Bioregenerative life support systems which utilize plants are being considered for this support. It is crucial, however, that we understand the influence of the space flight environment on the capacity of the plants to function properly. The plants will recycle water (transpiration), remove excess carbon dioxide and produce oxygen (photosynthesis), and produce food (growth and biomass partitioning). Therefore, results from studies such as this one will not only result in a more thorough understanding of the influence of adverse environmental conditions on primary plant metabolism but will also supply the information necessary to design and implement a space-based bioregenerative life support system with plants.

Publications, Presentations, and Other Accomplishments:

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Hilaire, E, JA Guikema and CS Brown "The fluid processing apparatus: From flight hardware to electron micrographs." J. Grav. Physiol., 2(1), 165-166. 1995.

Johnson, CF, TW Dreschel, CW Carlson and CS Brown "Analysis of water imbibition in a seed germination medium for the microgravity plant nutrient experiment on the KC-135." ASGSB Bulletin, 9(1), 48, 1995.

Piastuch, WC and CS Brown "The effects of chronic clinorotation on protein expression of *Arabidopsis thaliana*." J. Plant Physiol., 146, 329-332, 1995.

Sanwo, MM, GD Goins, NC Yorio and CS Brown "Effectof red light emitting diodes on photosynthesis and carbohydrate metabolism in superdwarf wheat plants." ASGSB Bulletin, 9(1), 50, 1995.

Sanwo, MM, NC Yorio and CS Brown "Spectral quality effects on growth and carbohydrate metabolism in wheat. In: Madore, MA and WJ Lucas (eds). Carbon partitioning and source sink interactions in plants." ASPP Press, Rockville, MD, 277, 1995.

Sanwo, MM, NC Yorio, BC Tripathy and CS Brown "Photosynthesis and carbohydrate metabolism in wheat plants grown under red light-emitting diodes." Plant Physiol., 108(2), 433, 1995.

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Yorio, NC, MM Sanwo and CS Brown "Growth and Carbohydrate metabolism of wheat plants grown with red light emitting diodes. " HortSci., 30(4), 863, 1995.

Physiological Anatomical Rodent Experiment (PARE) 04: Flight Support

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No Co-I's Assigned to this Task

Funding:

Project Identification: 5-01161

Solicitation: 93-OLMSA-03

Initial Funding Date: 4/94

Expiration: 4/96

FY 1995 Funding: $86,467

Students Funded Under Research: 0

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

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.

All ovaries have been serially sectioned, stained, and the ovarian morphometric analyses are completed. Pituitary LH and FSH content has been measured. The analysis of plasma concentrations of LH and FSH is complete, but we are still working on assays of the steroids. The analysis of the effects of space flight on post-implantation fecundity is finished. There was no effect of space flight during the post-implantation period on any of the ovarian morphometric parameters evaluated, nor on vaginal birth or fecundity. As a follow up to these studies, it seems imperative to investigate whether space flight initiated during the pre-implantation period has any effect on embryonic survival and fecundity.

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.

Publications, Presentations, and Other Accomplishments:

Burden, H.W. "Hypophyseal-ovarian function in rat dams flown on the NIH.R1 study." Annual Meeting of the American Society for Gravitational and Space Biology, 1995.

Burden, H.W., Zary, J., Lawrence, I.E., Jonnalagadda, P., Church, M., Hodson, C. "Hypophyseal-ovarian function in rat dams flown on the NIH.R1 study." American Society for Gravitational and Space Biology, Bulletin 9, 98 (abstract #172), 1995.

Effects of Space Flight on Muscles and Nerves

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-03

Initial Funding Date: 5/94

Expiration: 4/96

FY 1995 Funding: $98,203

Students Funded Under Research: 1

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: Ames Research Center

Task Description:

This project was designed to look at the role of gravity in the formation of skeletal muscles in the thigh of rats. Because skeletal muscles are derived from highly precise divisions through large pockets of precursor cells, it has for some time been of interest to understand the forces responsible for the control of the direction and timing of these divisions. To date, many possibilities have been eliminated as controlling factors, while none have been shown to play a role in development. Since the direction of these divisions is important, it was hypothesized that the force of gravity affecting all living things on the earth might be responsible for the signals dictating formation of muscles. If this is true, then animals developing in space, where the force of gravity is essentially eliminated, would be expected to have muscles that are different in shape or number, from muscles in animals that developed here on earth. This study will further our understanding of how muscles develop and may lead to advances in treatment of muscles following injury or disease.

A second question addressed in this study dealt with trying to understand the importance of force (gravity) on the development of the many proteins that make up skeletal muscle. During development, different types of proteins are present at different times. Because skeletal muscle proteins are affected by events that reduce the stress (force) on muscle (for example, bed rest or casting) we predicted that removal of the force of gravity during development would alter the way these proteins appear during development of muscle. Furthermore, since there is evidence from space flight experiments that recovery from injury is slowed down during space flight, we predicted that the development of proteins would be delayed in space compared to development on earth.

We received tissues from 20 fetuses that developed during space flight as well as 22 fetuses from the delayed synchronous control group that developed at Kennedy Space Center in cages identical to those on the space shuttle and under identical conditions of temperature and humidity. The tissues were fixed and sectioned, then treated with fluorescent probes that would delineate individual muscles in cross sections. The major results can be summarized as follows:

1. The general shape of the muscles in the thigh is not different following development in space compared to tissues that developed on earth.
2. Some subtle differences do exist between tissues that developed in space and those that developed on earth:
   a. The area between some muscles is larger following development in space compared to controls.
   b. Following development in space there are areas within particular muscles where a section of the muscle seems to
      be separate from the rest of the muscle.

It will be most informative to know if the spaces within muscles have been created by connective tissue or if they are really just space, and if these persist the entire length of the muscle. Continuing analyses of the tissues from this project are being performed to answer those questions. In addition it would be useful to get more tissues for analysis, both from experiments on the ground using different levels of gravity (for example, a 2G load put on the developing animals using the centrifuge at Ames Research Center), and from additional flight experiments with animals that develop for longer periods of time (for example, the entire gestation period) in space.

Ten micron thick sections from both fetuses and pups were used for the second experiment. This way, I could look at two time points during development to determine whether the process of protein development was totally different or delayed. These tissues were also fixed and sectioned, then treated with radioactive probes that would delineate individual proteins in cross sections of muscles. The major results can be summarized as follows:

1. The three different proteins (the RNA that will make the proteins) are all affected by development in space.
2. The three proteins were all affected differently.
   a. skeletal actin RNA is present to a lesser degree in fetuses from flight animals than from control animals. However, in thighs of pups, the RNA is present in similar amounts. This is consistent with our hypothesis.
   b. MLC RNA is present to a greater degree in fetuses from flight animals than from control animals. This is still true in the pups, but the differences are not as large as in the fetuses. These results are t he reverse of the hypothesis.
   c. MHC is not only present in greater amounts in the flight fetuses compared to controls, but the amount of RNA decreases in the flight group from the fetuses to the pups, while the amount of RNA increases from fetus to pup in the controls. This is quite different from the predicted results.

These results are exciting for several reasons. The first is that development in space affects protein development and this leads one to search for the possible reasons for those differences. The most likely reason is the lack of gravitational force on these developing muscles. Again, much more information could be gained by future collaborations with NASA using both the Centrifuge Facility at Ames Research Center and additional shuttle flights (or a space station) where tissues could be collected at more time points during development and animals could develop in space for the entire gestational period.

Another aspect of these results that is intriguing is that the three proteins were affected differently by development in space. Since these three proteins all function together to allow skeletal muscles to contract (and produce force), it is generally assumed that they are regulated by one or more similar factors during development. The results from this project would indicate that this might not be the

case. We are continuing to use the original tissues to look at additional levels of regulation to get a better understanding of what processes might be controlling protein development. Clearly the future studies could be highly beneficial in understanding how muscles develop.

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. This may lead to differences in treatment of muscle tissue following damage or disease.

Publications, Presentations, and Other Accomplishments:

Brown, D., Clark, K.I., Pellis, N.R., and Goodwin, T. "Characterization of skeletal muscle atrophy induced in simulated microgravity culture systems." In Vitro Cellular & Developmental Biology, 31 (3) pt 2, 1995.

Clark, K.I. Seminar, Center for Human Research, Dept. of Kinesiology, University of Michigan Medical School, Spring, 1995.

Clark, K.I. "Disorders of Voluntary Movement." Class Lecture: Kinesiology 610: University of Michigan Medical School, Spring, 1995.

Clark, K.I. Class Lecture: Anatomy 801: Graduate Student Seminar, Spring, 1995.

Clark, K.I. Special Presentation: Science Day, Young Astronauts Program, Ealy Elementary School, Spring, 1995.

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Clark, K.I. "Characterization of skeletal muscle atrophy induced in simulated microgravity culture systems." 1995 World Congress on In Vitro Biology, Spring, 1995.

Clark, K.I. Seminar: Students for the Education and Development of Space, University of Michigan Medical School, Fall, 1995.

Clark, K.I. "Research Methods." Class Lecture: MVS 421: Graduate Seminar, University of Michigan Medical School, Fall, 1995.

Clark, K.I. "Effects of spaceflight on development of neuromuscular systems." Meeting of American Society for Gravitational and Space Biology, Fall, 1995.

Clark, K.I. "Effects of spaceflight on development of neuromuscular systems." American Society for Gravitational and Space Biology , Bulletin 9, 173, 1995.

Clark, K.I., Brown, D. and Goodwin, T. "Role of tension in the development of skeletal muscle." In Vitro Cellular & Developmental Biiology, (in press).

Somatic Embryogenesis of Orchardgrass in Microgravity

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Funding:

Project Identification:

Solicitation: NRA-93-OLMSA-05

Initial Funding Date: 6/94

Expiration: 10/95

FY 1995 Funding: $63,158

Students Funded Under Research: 3

Flight Information:

Responsible NASA Center: Kennedy Space Center

Task Description:

The objective of the proposed research is to provide information on the influence of microgravity on embryo initiation, differentiation, development and the ultimate reproductive capacity of resultant plants utilizing an *in vitro* culture system in orchard grass (*Dactylis glomerata L.*) in which embryos initiate and develop from single mesophyll cells. Therefore the target cells remain *in situ*.

The system Is based on paired half-leaf segments which provides for a precise control and the opportunity to use paired statistics for the analyses of data. The experiments will provide information on quantity and quality of embryo formation, axis determination and polarity. Chromosome analysis will be conducted on somatic cells of regenerated plants and on meiotic cells of plants established in the field. Pollen fertility-sterility data will also be collected on field established plants as well as vigor and tolerance to stress, *viz.*, winter survival.

Our orchard grass (*Dactylis glomerata L.*) leaf culture system in which embryos initiate and develop from single mesophyll cells was utilized in the BRIC-O2 experiment flown 9-2O September 1994 on STS-4. The objective was to test the influence of microgravity on this system. The basal 30 mm of the two innermost leaves were split along the midvein, surface sterilized, and then rinsed with sterile water. They were cut transversely into segments of 3-4 mm length. The basal most four segments from the innermost leaf and the two most basal segments from the next leaf outward were plated onto Schenk and Hildebrandt medium amended with 30 mM dicamba. They were overlaid with Fluortex(TM) mesh (18OO mm pore size) to keep them in contact with the medium. Petri dishes containing segments from one leaf half were placed in canisters to be flown on the shuttle while dishes containing the corresponding "mirror" sections from the other leaf half were placed in canisters to serve as ground-controls. This procedure provides for a precise control and the use of paired statistics for the analyses of data. The leaf segments were plated 21, 14, 7, 3 and O.9 d (21 h) before launch. They were incubated in the dark at 21°C before the flight and for the remainder of a 4 wk callus/embryo induction period.

Somatic embryo formation was estimated by counting regenerated plantlets on each leaf segment in each petri (dish after transfer to medium without auxin and incubation for 39 (1 in 16 h light/8 h dark at 21°C/15°C. Somatic embryogenesis was significantly decreased from leaf segments plated 21 h, 3 d and 7 d prior to launch. It was only 30% of control in the 21 h treatment. The absence of gravity appears to strongly affect initial cell divisions and/or other early events leading to embryo formation.

Histological examinatlon of leaf segments plated 21 h before launch and fixed 3 h after landing indicated a higher ratio of anticlinal:periclinal divisions, reduced cell divisions, and a lower frequency of proembryo structures in the flight compared to the control treatment. Regenerated plantlets were established in a greenhouse. Tillers were selected from 75 treatment and 20 control plants and basal leaf tissue was used for chromosome analyses. Chromosomes were examined in approximately 40 metaphase cells per leaf base. All cells examined from control plants exhibited the normal chromosome complement of 2n=4x=28. Most of the regenerated plants from the fight treatment also had normal chromosome components. One regenerate was mixoploid, two others showed fragmented chromosomes, and several possessed separate cells with 15 to 56 chromosomes. Three hundred plants each from the flight and control treatments were established in the field. These will be used for pollen fertility-sterility studies and meiotic chromosome analyses.

The purpose of the research is to obtain additional and new information about the initiation and development of plant embryos Although the primary interest is the effect of the space environment on this process, results obtained should also have relevance to normal zygotic embryo development and seed formation. Data and observations from our flight experiment strongly suggest that the space environment, presumably microgravity, has strong negative effect on initial cell divisions or other early events leading to embryo formation. Since embryos are integral structures in seeds, an inhibition of embryo development would likely lead to a reduction in seed formation. This could be of extreme importance on a space station or long term space mission if there is a need to produce seeds, either for direct consumption, e.g., cereal grains, or for planting another generation of crops. Understanding the mechanisms or reasons for this reduction in cell division may allow us to learn more about cell structure, role of the cytoskeleton, etc., not only for cells involved in embryo formation, but for other somatic cells as well.

Publications, Presentations, and Other Accomplishments:

Conger, BV, Z Tomaszewski and JK MCDaniel "Effects of gamma radiation and microgravity on somatic embryogenesis from *Dactylis glomerata* leaf segments." 2nd Ukrainian Radiobiological Congress, Book of Abstracts, 1995.

McDaniel, JK, Z Tomaszewski Jr. and BV Conger "Histological studies of leaf tissue and chromosome analyses of regenerated plants from the BRIC-02 experiment with orchardgrass *in vitro* cultures." ASGSB Bull., 9(1), 93, 1995.

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Development of Sensory Receptors in Skeletal Muscle

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-03

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Expiration: 4/96

FY 1995 Funding: $87,542

Students Funded Under Research: 5

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: Ames Research Center

Task Description:

This study of rats, that undergo part of their prenatal development in space, will examine microscopically the formation of encapsulated sensory receptors - the two major types being muscle spindles and tendon organs - in hind limb skeletal muscles. It will determine the presence, number and size of the muscle spindles. This research also will test for any effects of space flight during late stages of gestation on the following events postnatally as the rats grow: weight gain, initiation of walking, eye opening, use of hind limbs during walking and ability to reproduce.

We have analyzed data collected on the pregnant rats and their offspring that were part of the STS-66 mission (NIH-R1 project) when they were alive, and we have studied microscopically some skeletal muscles from the offspring. Those data were compared to additional data obtained from control groups to reach the following conclusions.

Data collected from dams and their pups while they were alive provided several insights. In the first several hours after return to earth gravity, pregnant rats will be more inactive than earth-bound rats usually are. Space flight did not alter the integration of mechanisms that control postnatal weight gain in rats. Space flight did not interfere with the progression and sequence of two postnatal developmental horizons - initiation of walking and eye opening - in the rat. Space flight did not change the normal developmental progression for hind limb use typically seen during quadrapedal locomotion. Space flight exposure of pregnant rats does not preclude later subsequent reproductive capacity of their offspring, but it may result in lessened survivability of their own offspring and their offspring's own progeny.

Our preliminary observations show that muscle spindles and tendon organs do develop in a hind limb extensor muscle in rats exposed to near-zero gravity during much of the latter half of gestation and innervation of intrafusal fibers in those muscle spindles by both sensory and motor neurons does occur.

Encapsulated sensory receptors in skeletal muscles are important for the development and maintenance of normal somatic motor function. From an evolutionary perspective, encapsulated sensory receptors in skeletal muscle seem to have appeared when vertebrates became land dwellers. For example, muscle spindles and tendon organs are not present in 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 such as walking on a normal developmental schedule. 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 development of encapsulated sensory receptors in skeletal muscles of a mammal. Or said another way, at least in the short term fetal mammals 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.

Publications, Presentations, and Other Accomplishments:

DeSantis, M.E. "Sensory receptors in extraocular muscles of the camel." Fulbright scholar cooperative research award, Egypt/U.S., 1995.

DeSantis, M.E. "Vestibular system structure in adult rats after their prenatal development in a near-zero gravity condition." NASA Idaho Space Grant Consortium, U.S., 1995.

DeSantis, M.E., Wong, A. and Parkman, K. "Two motor horizons - walking and eye opening - are unaffected by prenatal exposure to near-zero gravity." American Society for Gravitational and Space Biology Bulletin, 9/1, 96, 1995.

Lindgren, J.A. and DeSantis, M.E. "Immunohistochemical demonstration of a stage-specific epitope in rat sciatic nerve during postnatal development." Developmental Brain Research, (submitted November 1995).

Parkman, K., Wong, A. and DeSantis, M.E. "Locomotor behavior of rats after exposure to the conditions of space flight." Journal of the Idaho Academy of Science, 31/1 , (in press).

Wong, A., Parkman, IK. and DeSantis, M.E. "Gender, more than space flight during part of gestation, influences postnatal weight gain in rats." American Society for Gravitational and Space Biology Bulletin, 9/1, 10, 1995.

Genetically Engineered Plant Biomonitors in Microgravity

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Funding:

Project Identification:

Solicitation: 93 OLMSA-05

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $119,566

Students Funded Under Research: 2

Flight Information:

Flight Assignment: PGIM-01 (STS-82, 1997)

Responsible NASA Center: Kennedy Space Center

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.

The TAGES biomonitor plants were carried aloft on the DC-9 research aircraft out of Lewis Research Center. *Arabidopsis thaliana* were genetically engineered with an essentially full-length alcohol dehydrogenase promoter driving the GUS reporter gene. Plant experiencing flights of 42-48 parabolas exhibited marked expression of the reporter gene, indicating the onset of aberrations similar to those reported from shuttle missions. The most dramatic expression of the reporter occurred in plants subjected to two flights in a single day.

These preliminary results offer proof of the concept of the original proposal: that reporter genes can be used to monitor the wide-range of alterations that space-flight experiences impart on plants. In addition, these results show that at least some of the early stages of the alterations that occur in space flight can be examined in atmospheric parabolic flights. This means that many problems involving space-flight stress perception can be approached or perhaps even solved before actual shuttle flights. Given the

positive preliminary results obtained from the initial parabolic flights, additional parabolic flights, as well as a series of centrifuge experiments, will be conducted in order to rule out possible effects due to the 2g portions. With these tests, we can advance our experiments and have it at the highest possible state of readiness for the orbital studies.

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.

Publications, Presentations, and Other Accomplishments:

Barbre, EC "Gators in space." University of Florida Today, 21, 10-15, 1995.

Chapman, DK, CJ Daugherty, HG LEvine, RJ Ferl, CS Brown and RM Wheeler "Biocompatibility tests with Plant Growth Facility prototype is compared to control plants in growth chambers." ASGSB Bulletin, 9, 51, 1995.

Effect of Spaceflight on the Development of the Circadian Timing System

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Funding:

Project Identification:

Solicitation: 93 OLMSA-03

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $215,000

Students Funded Under Research: 10

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

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 proposal 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. (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 the fiscal year 1995 of the NASA Grant, Effect of Space flight on the Development of the Circadian Timing System, several of the critical tasks have been accomplished. During this time we have verified the protocols and procedures that were tested during PVT. The tissue was shown to be adequate for the immunohistochemical protocols. In addition, we established some important developmental markers for the retina and SCN. We demonstrated that development of c-Fos expression within the retina and SCN following a phase shifting light pulse occurs between postnatal day 3 and 5. We also verified the use of the biotelemetry implants to record the development of body temperature and activity circadian rhythms in PN17 rats.

We had a successful launch, flight, and landing of 10 pregnant rats aboard STS-70. All pregnant flight and control rats had successful births at the anticipated time. All of the experimental protocols for histological preparation went well. All the procedures for shipping of tissue and rats to UC Davis were successful. We have been processing the retinal and brain tissue, and are currently analyzing the retinal and brain tissue from the G20 rats stained for c-Fos and apoptosis. Other ages will be analyzed as the tissue for the various postnatal ages is processed.

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 the animal. For example, the conditions of 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 hyperdynamic fields via centrifugation significantly affects the CTS. In addition, mature animals exposed to the microgravity of space flight exhibit altered circadian function. Although previous studies have demonstrated that exposure to space flight 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 proposal will examine 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.

Publications, Presentations, and Other Accomplishments:

Hoban-Higgins, T. M., D. M. Murakami, C. Fermin, and C. A. Fuller "Development of circadain rhythms of body temperature and activity in Sprague-Dawley rat pups." Soc. for Neurosci., 21, 955 (1995).

Murakami, D. M., I.-H. Tang, T. M. Hoban-Higgins, and C. A. Fuller "The development of the photic induction of c-Fos in the retina and SCN." Soc. for Neurosci., 21, 956 (1995).

Effects of Hypogravity on Osteoblast Differentiation

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Funding:

Project Identification:

Solicitation: 93 OLMSA-04

Initial Funding Date: 2/94

Expiration: 12/95

FY 1995 Funding: $37,943

Students Funded Under Research: 0

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-C3 (STS-63, 2/95)

Responsible NASA Center: Ames Research Center

Task Description:

Weightbearing is essential for normal skeletal function. Without weightbearing, the rate of bone formation by osteoblasts decreases in the growing rat. Defective formation may account for the decrease in the maturation, strength and mass of bone that is caused by space flight. These skeletal defects may be mediated by a combination of physiologic changes triggered by space flight, including skeletal unloading, fluid shifts, and stress-induced endocrine factors. The fundamental question of whether the defects in osteoblast function due to weightlessness are mediated by localized skeletal unloading or by systemic physiologic adaptations such as fluid shifts has not been answered. Furthermore, bone-forming activity of osteoblasts during unloading may be affected by paracrine signals from vascular, monocytic, and neural cells that also reside in skeletal tissue. Therefore we propose to examine whether exposure of cultured rat osteoblasts to space flight inhibits cellular differentiation and impairs mineralization when isolated from the influence of both systemic factors and other skeletal cells. Growth of primary fetal rat osteoblasts on microcarriers enhances differentiation of osteoblasts and mineralization of the collagenous matrix. Using this culture system, we intend to address the question of whether space flight directly affects the differentiation of osteoblasts.

Results from STS-59 (NIH-C1) revealed that glucose utilization during space flight was significantly lower than ground control cultures, and the production of lactate concomitantly decreased. In addition, ultrastructural analysis by electron microscopy revealed that osteoblasts exposed to space flight possessed a smaller amount of well-organized, rough endoplasmic reticulum/Golgi apparatus than ground controls. This result indicates that space flight may inhibit the secretory activity of osteoblasts, a fundamental requirement for new bone formation. Thus, space flight may regulate both energy metabolism and the differentiated function of osteoblasts.

Control and flight cell cultures on STS-63 (NIH-C3) acquired a bacterial contamination in the course of the experiment, and therefore data acquired from this flight are not informative.

Before the mechanisms of weightlessness and disuse-induced inhibition of bone formation can be understood in detail, it is important to establish whether space flight alters bone formation when osteoblasts are isolated from systemic endocrine influences. The preliminary results from this study reveal that space flight does indeed directly affect the function of cultured osteoblasts. These experiments laid the groundwork for future studies that will address the mechanisms involved in sensing gravity, a basic biological process that is not yet understood.

Publications, Presentations, and Other Accomplishments:

Doty, S.B., A. Boskey, I. Binderman, R.K. Globus, and E.M. Holton "The effect of spaceflight on bone and cartilage cell differentiation." 5th International Conference on Mineralized Tissues, Oct. 22, 1995.

Doty, S.B., Boskey, A., Binderman, I., Globus, R.K., and Holton, E.M. "The effect of spaceflight on bone and cartilage cell differentiation (Abstract)." 5th International Conference on Mineralized Tissues, October 22, 1995.

Globus, R.K., Mourse, A., Zimmerman, D. Lull, J., and C. Damsky "Integrin-extracellular matrix interactions in connective tissue remodeling and osteoblast differentiation." Amer. Soc. for Gravitational and Space Biology Bulletin, 8(2), 19-28, 1995.

Malouvier, A., Globus, R.K., Doty, S., Lull, J., and Morey-Holton, E. "Gravity regulates glucose and lactate metabolism in cultured osteoblasts" (Abstract). ASGSB Bull., vol. 9, 28, 1995.

Effect of Microgravity on Epidermal Development in the Rat

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Funding:

Project Identification:

Solicitation: 93 OLMSA-03

Initial Funding Date: 6/94

Expiration: 5/96

FY 1995 Funding: $31,432

Students Funded Under Research: 2

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: Ames Research Center

Task Description:

The effects of space flight and microgravity on the multiple functions of the skin has not yet been explored. This research will examine the composition, organization and integrity of the skin rats develop under the conditions of space flight. Analysis will include: the amount of calcium in the skin; a microscopic look at the cellular organization of its outermost layer; and measurement of selected properties. The data obtained from these studies will result in a better understanding of the effects of nonterrestrial environments in altering the development and maturation of skin.

The task progress was completed in this funding year. There were several significant results of this project:

Pregnancy in the Sprague-Dawley rat can be maintained under the adverse conditions of space flight and readaptation to terrestrial gravity;

No evidence of increased fetal wastage or somatic growth retardation was observed;

Vaginal delivery can be achieved following short-term (e days) readaptation to terrestrial conditions;

Epidermal barrier development in the late gestational fetal rat appears to be advanced under the conditions examined;

Fetal skin calcium levels are increased following development under conditions of microgravity;

Neonatal epidermal calcium levels are decreased following short term readaptation to terrestrial gravity;

Morphologically the epidermal barrier is advanced by 12-24 hours;

Measurement of water flux and electrical resistance of the skin support the hypothesis of a better epidermal barrier in the flight animals compared to ground controls.

The epidermis forms the ultimate bioevolutionary "space suit" interfacing the human organism with his physical environment. Non-invasive instruments are currently available for quantitating physical properties of the outermost layer of the skin (the stratum corneum). Such instrumentation includes devices for measuring surface acidity, water content, hydrophobicity, viscoelasticity, frictional coefficient, desquamation indices and other important surface properties. The development of better non-invasive instrumentation for assessing skin surface physical properties may be a legitimate area to pursue in order to reach NASA objectives. For example, the assessment of physiologic state during and following extra-vehicular activity (EVA) may be enhanced by skin-based monitoring devices (temperature, blood flow, transepidermal water). Such NASA-based sensing systems may find parallel application in biomedical settings (for example, physiological monitoring in intensive care units). Studies in humans focusing on the epidermal-environmental interface are: (1) feasible given the easy accessibility of the epidermis, (2) developmentally relevant given this tissue's biological property of continual cellular replacement and, (3) practical given the important role of the stratum corneum as a platform for non-invasive physiological monitoring.

Significant advances may accompany the promotion by NASA of skin research. The concept that the skin forms the natural "space suit" for the body is an easy one for the public to grasp. From a biological standpoint, the skin is complex and dynamic organ which is high adaptive to changes in environmental conditions. The presence of a self-replenishing boundary layer with sensing capabilities fits neatly into the field of "smart materials" research. The strategic location of the skin between the body and the external environment (outer space) makes it a logical target for information retrieval technologies. The development of new sensing systems using skin-based techniques should have practical spin-offs to the medical care environment as well as the skin care industry. The re-application of NASA developed technologies would be expected to have a positive impact on future agency funding.

Publications, Presentations, and Other Accomplishments:

Hoath, S.B., W.L. Pickens and A. Hussain "Effects of spaceflight and readaptation on epidermal barrier development in the fetal rat." American Society for Gravitational and Space Biology Annual Meeting, October 25-29, Arlington, VA, 1995.

Regulation of Cell Wall Polymers by Microgravity

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Funding:

Project Identification:

Solicitation: 93 OLMSA-05

Initial Funding Date: 6/94

Expiration: 10/95

FY 1995 Funding: $146,000

Students Funded Under Research: 4

Flight Information:

Flight Assignment: CHROMEX-6 (STS-63, 2/95)

Responsible NASA Center: Kennedy Space Center

Task Description:

Our hypothesis stated that the modifications in plant cell walls during growth in microgravity are due to alterations in cell wall architecture, specifically altered cell wall extensibility. Extensibility may be governed by the number and type of cross-linkages in the wall. Key enzymes involved in cross-linking the structural polymers are peroxidases. We proposed to determine whether peroxidases are involved in altered cell wall extensibility. If this relationship is found, genetic modification of the peroxidase(s) may allow modifications that permit normal growth in microgravity. Microscopic and biochemical analyses of the cell walls of a superdwarf wheat cultivar developed for growth in space were conducted under space flight conditions to elucidate the response to gravity.

Data collection for this project has been completed, and currently the data are being analyzed and summarized into final publication form. Gas sampling results showed that before launch, levels of CO^2 ranged from 700 to 1300 ppm (0.07-0.13%) in the chambers. These levels were 2.3-4 times higher than the 0.03% atmospheric level, indicating that the chambers were already accumulating CO^2 during the 2-3 h post loading and sealing period, before pre-flight samples were drawn. The inflight and ground control chambers were sampled post flight. In ground control chambers, CO^2 increased to approximately 80,000-150,000 ppm (8-15%), levels 250-500 fold above ambient. The chambers with living plants from the Shuttle (PGCs 2, 4, 5, and 6) also accumulated high levels of CO^2 (140,000-160,000 ppm).

Ethylene in flight and ground control PGCs were below 16 ppb, i.e., at ambient levels, prior to flight. Ground control chambers accumulated 1 to 2.7 ppm ethylene, at the lower end of the range of biologically active concentrations. These levels of ethylene were a sum of 10 days of accumulation in the sealed chamber, indicating that on any single day, the accumulation was not more than 0.1 to 0.27 ppm, assuming a linear accumulation. Again, PGCs 3 and 6 contained lower concentrations of

ethylene, suggesting that these chambers were not tightly sealed. In contrast, no flight PGC accumulated ethylene above 70 ppb (0.07 ppm), suggesting an inhibition of ethylene synthesis or evolution under microgravity conditions. If microgravity is considered a stress, one would expect ethylene levels to increase when plants were subjected to this perceived stress. However, it appeared that the gravity experienced by the ground control plants was the source of stress that caused ethylene production. Thus ethylene did not appear to be a factor in producing the results discussed below.

Apoplastic sap enzymes analyzed were peroxidases. They were separated on 1.2% agarose gels with 18% sorbitol and ampholytes (Pharmalyte) in a pH range of 3.0-10.0. Gels were run for 1-1.5 hours at constant power (approximately 8W, 500V). The staining reaction contained 0.2% catechol and 0.1% p-phenylenediamine in 0.01M Tris pH 7.5. The reaction was initiated by adding 30% hydrogen peroxide to a final concentration of 0.09%. Proteins with peroxidase activity appeared as reddish-brown bands in the gel because of localized oxidation of the substrate. The reaction mixture for activity in extracts contained 3.0 mL potassium phosphate buffer (0.01M), pH 6.0; 0.04 mL hydrogen peroxide (0.03M), 0.05 mL guiacol (0.2M), 0.005-0.02 mL cell wall extract. The reaction was initiated by vortexing with wall extract. 0.1 mL of reaction mix was transferred to a microcuvette and absorbence at 470 nm recorded at 1 minute intervals, starting 30 seconds after reaction initiation. Specific peroxidase activity was expressed as the change in absorbence at 470 nm over one minute (linear phase) per microgram of protein. Peroxidase activity tended to be higher in microgravity grown plants. In addition, a unique peroxidase isozyme, pI-5.7 was detected in extracts from microgravity-grown roots.

Plants were stained with toluidine blue for anatomy, phloroglucinol for lignin and starch/iodide for peroxide. Anatomy appeared to be normal at the light level. Lignin accumulated in all leaf and root tissues of space-grown plants. This was a surprising result considering literature stating the loss of total lignin, protein and cellulose in space flight. However, because this was only an 8-day mission, perhaps longer term flight would affect this result. Also, the lignin accumulation correlates well with the appearance of increased peroxidase activity. The peroxidase staining in leaves and roots of space grown plants appeared less diffuse than in ground controls, suggesting it was being used for lignin formation and deposition.

For electron microscopy, ten areas of three plants from three chambers were free-hand sectioned (250-300um) and mounted for cryofixation. Samples were cryo-fixed in a LifeCell CF100 Cryofixation apparatus provided and operated by personnel from Research Manufacturing Inc. (RMC). Samples were distillation dried, fixed and embedded using a Console Molecular Distillation Dryer (MDD-C), a service performed by Victoria Hatch, Physiology Program, Harvard School of Public Health. Multiple ultra-thin sections of each block were viewed for ultrastructure and analyzed for Ca^{2+} localization. Ca^{2+} localization was accomplished through electron energy loss spectroscopy (EELS) on a Zeiss CEM902 transmission electron microscope. Preliminary results are inconclusive for Ca^{2+} localization. Cell walls appear to be thinner in tissues from microgravity flown plants.

Five to six plants from each chamber were cut at the shoot/root junction. Each shoot was measured from the cut surface to the tip of the longest leaf; each root from the cut surface to the tip of the longest root. The mass of each piece was determined on a milligram balance. Means and standard errors were determined for each chamber and for the entire population from either space flight or ground controls. In ground controls and microgravity grown plants, shoot length and mass were quite similar. Interestingly, however, roots tended to be longer and root mass less in the microgravity grown plants compared to ground controls. The standard errors of the root length and mass measurements did not overlap, making the differences significant, i.e., the trend was definitely toward longer, less massive roots from microgravity-grown plants. From these data, the conclusion may be drawn that leaves and pre-stems (i.e. leaf sheaths) show similar growth responses plus and minus gravity.

Leaves, leaf sheaths (pre-stems) and root pieces were placed in bundles overnight at $40^{o}C$ to dry. Composite strength (extensibility or tenacity) of walls was determined by stretching plant parts in an Instron. Material was clamped without stress, then weights were released and clamps pulled apart until

the plant material broke--the peak height of tenacity at breakage was recorded. All material between the clamps was recovered by severing it at the clamp edges with a razor blade. The material was weighed and tenacity calculated as grams of resistance to breakage per mg dry weight of tissue. Leaves and leaf sheaths from microgravity-grown and ground control plants had similar lengths--averaging approximately 0.0025 g/mg (leaves) to 0.003 g/mg (leaf sheaths). The most interesting comparison was between roots of ground controls and of microgravity-grown plants. Microgravity-grown root tips tended to be stronger that ground control root tips. These data correlated with the increased staining for lignin observed in all root regions of microgravity-grown plants compared to ground controls. However, when the data were viewed more closely, two populations of roots were observed--those showing increased strengths over ground controls and those with strengths similar to ground controls. These data suggest that flight roots may have been responding differently to the removal of gravity based on some other criterion for which we did not or could not control. This could possibly have been something as simple as whether the roots measured were contained within the floral foam or grew aerially in the chamber. Future experiments should explore this phenomenon more closely. Carbohydrate analyses for neutral sugars were conducted on crude cell wall preparations and results were inconclusive.

The results suggest further studies in which one of these observed alterations--lignin staining, peroxidase activity or isozymes, extensibility, morphology or ultrastructure--may be used to analyze the sensing and response mechanisms for gravity. Transgenic plants in which a gene whose product will affect any of the observed structural molecules would provide more definitive answers to the sensing of and response mechanism to the gravity stimulus. Plants such as these could be grown in hyper- and micro-gravity. Ageotropic plants transgenic for the altered traits observed here would also be useful.

Earth benefits of this research do not directly relate to human disease or physiology. This study was directed toward understanding the development of plants in microgravity. Gravity is an environmental condition that is always present in the life of a plant, and it exerts a significant influence on plant growth and development. Answering the question of how plants respond to gravity is a challenge because removing the influence of gravity is difficult. Understanding how plants respond in reduced gravity helps us understand the processes of their development in normal gravity. This will help scientists understand how to modify crop plants for Earth-based or space-based agricultural systems to maximize production from limited areas.

Publications, Presentations, and Other Accomplishments:

Bishop, DL, HG Levine, WR McManus, A. Singh-Cundy, and EE Hood "Wheat cell wall structure in microgravity. II. Lignin deposition, hydrogen peroxide concentration, calcium localization and cell wall anatomy from CHROMEX-06 Experiment; STS-63." ASGSB Bulletin, 9:92 #157, (1995).

Hood, EE, DL Bishop, HG Levine, A Singh-Cundy, and HZ Lanouve "Wheat cells wall structure in microgravity. I. Plant growth parameters, tensile strength and carbohydrate analysis from CHROMEX-06 Experiment; STS-63." ASGSB Bulletin, 9:92 #156, (1995).

Singh-Cundy, A, DL Bishop, and EE Hood "Wheat cell wall structure in microgravity. III. Peroxidase activity and protein profiles in soluble cell wall extracts from CHROMEX-06 Experiment; STS-63. " ASGSB Bulletin, 9:92 #158, (1995).

Effect of Gravity on the Attachment of Tendon to Bone

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Funding:

Project Identification:

Solicitation: 93 OLMSA-03

Initial Funding Date: 6/94

Expiration: 4/96

FY 1995 Funding: $

Students Funded Under Research: 1

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

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 a 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.

To date, we have received and processed all samples for either light or scanning electron microscopic analysis and have completed nearly 75% of the histomorphometric analysis. We have characterized the changes caused by space flight to tendon attachments to the tibia, fibula and femur. We have not yet determined the effects of space flight on the calcaneus nor measured atrophy of muscles attaching to the femur, tibia and fibula. Our results suggest severe osteoporosis in the femur, fibula and tibia of animals coincident to space flight, which had not resolved after 4-5 days following return to Earth. This

was evident at all sites, including sites of tendon attachments. Comparison of scanning photomicrographs of flight animals with other lactating animals demonstrated structural similarities and suggested that it might be worthwhile to assess whether lactation is a factor in development of the osteoporosis in the space flight animals. In addition, evaluation of total calcium utilization by space flight animals would be beneficial.

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 but its metabolism is much more rapid than 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.

Publications, Presentations, and Other Accomplishments:

Johnson, R. B.; Tsao, A. K.; St. John, K. R.; Betcher, R. A.; Tucci, M. A.; Parsell, D E..; Mushell, N.; Zardiackas, L. D.; and Benghuzzi, H. A "Effects of spaceflight on the attachment of tendons to bone." Amer. Soc. Gravitational and Space Biology, Bulletin 9, 97 (1995).

Plant Embryos and Fidelity of Cell Division in Space

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-05

Initial Funding Date: 10/95

Expiration: 9/96

FY 1995 Funding: $79,735

Students Funded Under Research: 2

Flight Information:

Flight Assignment: BRIC-08 (STS-77, 4/96)

Responsible NASA Center: Kennedy Space Center

Task Description:

Dr. Krikorian will test whether the cell division changes observed in the daylily 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 will house 27 petri dishes of daylily cells in an agar type medium,
- there will be no inflight manipulation,
- upon landing, 85% of the cells will be chemically fixed for examination while 15% will be allowed to develop.
- ground controls will be developed in parallel to the flight experiment

Studies to enhance performance of the experimental system and to optimize test features that are expected in the course of flight have been the focus of the ground work in the pre-flight period.

Included in this is a continued effort to improve and streamline all aspects of the experiment system, including complete characterization analysis and evaluation of performance of test embryogenic cell materials under differing regimes of nutrition and environment.

(1) Refinement of various steps towards an efficient embryogenic cell culture system for daylily for exploitation in space biology work focusing on karyological analysis have now been realized.

Several new embryogenic cell cultures of daylily with different nuclear status (chromosomal profiles) have been initiated for the express purpose of the upcoming flight. An important component of plant cell cultures, especially those that are comprised of embryo initials such as the ones (which may be termed "embryocytes") to be used in the upcoming experiment is to have vigorous cultures that are well-characterized. Since these cultures cannot be purchased, it is one of our on-going activities to initiate, monitor and manage embryogenic cell suspensions so that they are available for needed experimentation.

Since initiation of embryogenic cultures is a process (in contrast to an event), considerable skill must be exercised in establishing these. A minimum of six months' lead is needed prior to scale-up of cell materials. Diploid and tetraploid cultures are available and are being evaluated (1) for their chromosomal stability and (2) for the quality of their phenotypic embryogenic progression. Only cultures which meet the highest criteria on both accounts are retained for multiplication and maintenance and study. Even so, the features of those cultures which do not meet our expectations are quite valuable from an instructional viewpoint and are entered into our data base of fidelity of response (or lack thereof).

(2) Gas mixtures of differing composition are being tested for their impact on the growth and performance of somatic embryos.

At the time of this writing a gas mixture of 5 % carbon dioxide, 25 % oxygen and 75 % nitrogen are envisaged as the best composition for the "ambient air" to be used in the BRIC environment.

It has been appreciated for some time that gaseous contaminants have the potential to interfere with cell growth and development in the space environment. Stated another way, it has not been demonstrated that gaseous pollutants present in the space environment do not have an adverse effect on plant growth and development in space. In fact, there are many possibilities for a series of indirect effects to be brought into play as a plant embryogenic or any other system develops and grows in space. We are trying to eliminate the possibility that perturbation of the gaseous environment is responsible for some of the cytological anomalies that we have encountered. Ground work done some time ago failed to disclose adverse effects of ethylene on somatic embryogenesis process itself. However, the means to establish critically that intracellular levels (in contrast to levels exogenous to the tissues) of ethylene are not increased are not yet available. So it remains a moot point whether ethylene gas is as innocuous as it seems.

Our overall working hypothesis acknowledges that there may be subtle effects due to lack of convective currents (which would affect both heat or gas exchange), change(s) in buoyancy of fluids associated with vital processes, e.g. dissolved gases easily come out of solution during fluid flow, and change(s) in space in physical properties of fluids such as the pronounced increases in surface tension. (These could affect interfaces in or surrounding living tissues, e.g. effects due to Marangoni convection, that special convection which is induced by surface tension differences.) Water behaves differently in space in a number of ways: water suspended in a gas attracts itself and appears as a sphere; once water 'wets' a surface, it quickly spreads out and flows in all directions along that surface, etc. The intention is that as opportunity permits, each of these will be addressed, either singly or in various testable combinations.

All of our previous flight work has been done in dishes that have been either open to the middeck locker or ambient cabin atmosphere. For instance in the J mission (two NASDA-type dishes were 'flown'), the cabin air was filtered through an opening which permits aseptic ventilation via a 25 mm diameter TF-45O Gelman filter (HT45O Gelman No. 66221 actually used) located in the lid of the dish. Again, in the IML-2 experiment, again using NASDAtype dishes of the kind that could not be used for chemical fixation, along with some that could be used for chemical fixation, a 25 mm diameter TF-45O Gelman filter (HT-45O Gelman No. 66221) was used as well. This filter is essentially a HEPA filter that does not have the capability of excluding gases. In the BRIC experiment performed last year, off-the-shelf, Nalgene polycarbonate, lOO x 15 mm (Nalgene Brand Cat # 55O2-OOlO), petri dishes

were utilized. The BRICS, while sealed with a lid and an o-ring, accommodated nine (9) such dishes arrayed in a stack, were not air-tight.

The decision was made to design a BRIC so that it would be possible to flush the airspace with gas mixtures of known composition. The BRIC would thus have to be "leak-proof" to gases. Preliminary tests at KSC were carried out and it was learned that the canisters held gas for at least a week, suggesting that the BRICS were "pretty tight". Four (4) BRICS that can accommodate 4 plastic petri dishes each have been made available to us at Stony Brook for ground testing. The seal is quite innovative and seems to minimize if not eliminate leakage. Tests using Freon gas to evaluate leak rate are in progress. These are being done in cooperation with the refrigeration shop in the Life Sciences Division and should provide a good quantitative measure of the seal. This information will serve a double purpose in that it will be important to know how long a good seal can be maintained in the BRICS.

(3) Specific Ground Testing using the BRIC Canister System

Nine (9) BRIC canisters that can accommodate 4 plastic petri dishes each are planned for the flight this summer as a mid-deck experiment. The dishes are available off-the-shelf and made of Nalgene polycarbonate, lOO x 15 mm (Nalgene Brand Cat # 55O2-OOlO). These are reusable and autoclavable. No honeycomb inserts will be used to prevent, as a precautionary measure, dislodging of the semi-solid substrate. We have learned that honeycomb inserts are not needed to keep the agar in place in space, and there may even be some associated toxicity [in the USA this material is made of an aromatic amide (PN2 Aramid Fiber Honeycomb Core) which has the DuPont trade name of NoMEX]. Activated Charcoal-Impregnated Filter Paper Circles in Petri Dishes (# 5O8, Schleicher and Schuell, Keene, NH) will be placed on the culture medium. The papers fit pretty well in the lOO mm dia plastic petri dish and need not be cut or modified.

Because the embryogenic cell set-up entails use of activated charcoal filter paper, and dialysis membrane placed on top of the culture medium, there is considerable degree of confidence that any potentially toxic substances that could have adverse effects on growth or cytology would be adsorbed and thereby presumably inactivated or rendered less accessible to the developing embryogenic cells. This claim is further substantiated by the fact that none of our ground controls have shown any cytological aberrations even in those instances where honeycomb inserts have been used.

(4) one of the main points of the flight experiment is to test the hypothesis that space-related stress due to the way water behaves in space is a prime cause of the curtailment of embryo growth in space and also the main cause (in combination with other space stresses) of our chromosomal abnormalities.

My supposition is that (a) cell function and behavior of embryogenic cells (embryocytes) will be normal in space/microgravity through all the essential stages of embryogenesis and into a miniature plantlet in a lOO mm diameter petri dish in the BRIC set-up provided they are sufficiently protected from stress, i.e., appropriate-stage embryo initials are embedded in an appropriate and defined semi-solid substrate nutrient medium that it is optimized for their progression. The conditions need to be carefully adjusted so as to render the embryogenic process resistant to a range of factors generically referred to as stress effects. A matrix of test variables involving level of embryogenic cell complexity and embryogenic stage, DNA level status as reflected by chromosome complement, i.e., ploidy level, and water potential continues to be tested so as to probe interactions, and to achieve the right set of parameters that reflect "optimization". (b) Conversely, somatic embryogenic initials (embryocytes), will not progress without signs of cytological or morphological stress if they encounter a severely compromised "stressed" growing environment. Embryogenic units that are less developmentally advanced will show stress effects while those that are more advanced will not. Polyploid (tetraploid) lines will be more tolerant of stress and diploid lines will be less so. In the context of (a) and (b), and for the purposes of the specific design, "optimized" here refers to proper unit size, embedded in an appropriately prepared semi-solid nutrient medium, and "stressed" refers to inappropriate size and/or on

a membrane on the same semi-soiid nutrient medium. The difference is that those of insufficient level of advancement on have a potential to become water stressed (i.e., in this case, too much water not, as is usually thought of, too little). The "too much" water can be imposed on Earth by inadequate osmotic priming or "drying". In space or microgravity, too much water derives from the fact that water vapor in sealed dishes will condense in microgravity on the "agar-gel" surface and prevent normal embryogenic progression even though the cells were primed to progress on earth by proper osmotic adjustment! A major task has been to evaluate the precise means to achieve the proper level of agar 'hardness' in the test systems.

Our efforts to maximize the regenerative response have emphasized the importance of the water relations during culture. The water potential of an environment is defined as the sum of the matric, pressure, and osmotic potentials. Growth on agar medium creates the most complex environment with respect to water relations. The water relation of cellular material with respect to the matric component of a solid surface can be temporally quite drastic. The matric component of the surface may exert a strong negative potential initially, then increase to a more moderate value as the tissue as a whole distances itself from the surface. In non-saturated states, the matric potential of filter papers, wood, soil, gelatin, agar, clay, etc., can be as low as -3OO MPa. Additionally, as would be the case for the embryogenic initials, the matric potential is of special importance for keeping water out in tissues with cells where the vacuole(s) are small. Also, when tissue is plated on to a semi-solid surface such as agar it must be remembered that there is another exposed area with a low water potential, namely air. This value depends on the temperature and relative humidity.

For embryocytes undergoing regenerative somatic embryogenesis, correction of the water potential of the environment to that which would be found in a seed at that particular developmental stage has seemed a logical starting point. It is known that the endosperm has the lowest (most negative) osmotic value (-l.O MPa.) followed by the embryo, the seed coat and the pod tissues. During the cotyledon stage, water and osmotic potentials are lowest in the embryo proper, decreasing to -2.O MPa. This reflects transfer and synthesis of food stores. The routine but empirical use of increased osmoticum physically applied for regenerating small units and single cells, is commonly found in the literature.

Outside of a physiological requirement, the significant contribution that drying may bestow upon the regeneration phase of daylily somatic embryogenesis is the reversal of a vitrified state, which is acquired in the induction phase. Vitrification, more recently referred to as "hyper-hydricity", is not in all cases simply due to an excess of water, as this condition might be symptomatic of an underlying metabolic perturbation. Cytokinin and M + S have been linked to vitrification. That drying is the ultimate remedy to vitrification is supported in work done with artichoke which aimed to evaluate the value of different treatments used to overcome vitrification. In this study, the only way to overcome vitrification in tissue culture-derived artichoke was by raising the agar concentration of the medium. It was concluded that the matric potential was responsible for this phenomenon.

Agar is a complex mixture of polysaccharides extracted from species of red algae. Agar yields two polysaccharide fractions:1) A virtually neutral polymer, agarose (1-4)-linked 3,6-anhydro-c-L-galactose alternating with (1-3)-linked-D-galactose; 2) A charged polymer, agaropectin, having the same repeat units as agarose, with some of the 3,6-anhydro-L-galactose residues replaced with L-galactose sulfate residues, together with partial replacement to the D-galactose residues with pyruvic acid acetal 4,6-o-(lcarboxyethylidene)-D-galactose.

Agarose is the component responsible for the high-strength geling properties of agar, whereas agaropectin provides the viscous component. Thus, the gel strength of an agar is related to the percentage of agarose present in relation to agaropectin.

A major problem with agar is that its properties vary with the biological source, the way it is produced and processed and bleached etc. Manufacturers are not very consistent with the quality control and from time to time, properties vary considerably. I have attempted over the years to standardize the source of

agar and have purchased bulk quantities to maintain consistency. Moreover, we have developed our own washing procedures.

The main feature of agar for us in the BRIC experiment is to have gel strengths of varying levels in the final medium. The gel strength is sometimes referred to as Grade Strength and bacteriological agars for example range from 145 to 190. By using different mixtures of agar and Gelrite we have come up with a range of gel strengths that can give the system the best chance to be stressed versus not stressed. The final exact strengths are in the process of being decided. Since nine (9) BRIC canisters will be available, and each will hold 4 petri dishes, the plan is to use each BRIC to house a single parameter of gel strength.

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 this 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, 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 lg. Any changes detected in altered growth characteristics or disturbed development in microgravity will be correlated at the cell, tissue and organ level and further detailed at the biochemical and molecular level. 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" they 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 is 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.

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Molecular and Cellular Analysis of Space Flown Myoblasts

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Task Description:

While many of the overt physiological effects of microgravity can be compensated for by various countermeasures, effects at the cellular and molecular levels may require other means of intervention. However, little detail is known about the direct effect of microgravity at the molecular and cellular level. Insight into the cellular and molecular events responsible for muscle cell growth and development come in large part from *in vitro* studies with established cell lines. This investigation will use a well characterized rat skeletal muscle cell line, in the Space Tissue Loss-A (STL-A) module. The specific goals of the muscle cell culture model are to augment the whole animal model studies and simplify the molecular and cellular analysis of microgravity effects on muscle tissue in general.

For Dr. Kulesh's research, rat muscle cells will be cultured in individual cell cartridges and sustained in the STL module. The experiment itself is passive, requiring no in-flight manipulation except for temperature monitoring. The experiment requires special preparations before launch and immediate removal from the Shuttle after landing, to access the effects of microgravity on the growth of muscle cells, before the effects of full gravity are reestablished.

Post-flight experiments with the space flown muscle cells will evaluate the overall effect of microgravity on cellular characteristics (shape, doubling times, etc.). In addition the investigator will begin to assess possible changes in the expression of proteins and genes after their exposure to microgravity.

Gravity may play an integral role in the biological functioning of single cells. Information on the effects of gravity on muscle cell development will help scientists overcome the deleterious effects of space travel. These studies in weightlessness will also contribute to the understanding of cell proliferation, cell differentiation, development and wound healing.

During 1995, we have begun an in-depth analysis of L8 cells from both STS-45 and STS-63 which included: A) Flight Log and Cell Culture Results from STS-63; B) Repeated labeling indices analysis on STS-63 L8 cells which were initially performed on the L8 cells from STS-45; C) Continued an analysis of sarcomeric myosin, and actin, desmin, vimentin and titin immunofluorescent antibody staining patterns in the STS-45 and STS-63 L8 cells; D) Began an analysis of the expression of muscle differentiation genes (MyoD, MRF4, myf-5, myogenin and ID (Inhibitor of differentiation) in both the STS-45 and STS-63 cells.

The STS-63 mission of Shuttle *Discovery* occurred Feb 3 - Feb 11, 1995. The STS-63 STL-A unit contained four NASA-sponsored experimental groupings from Harvard University and NASA Ames Research Center involving primary bone cells; Naval Research Medical Institute (NMRI) involving stem cells; and Armed Forces Institute of Pathology (AFIP) L8 rat myoblast cells.

Preflight activities for STS-63 included four separate cultures of L8 myoblast cells (2 carried from AFIP and 2 thawed upon arrival at the NASA Launch Site Support Facility (LSSF)) that were constantly monitored and maintained in preparation for loading the cells into both the ground control and flight cartridges. Cell culture manipulations and various flight preparations during this time went without incident - good cell growth and no detectable contamination. A preliminary cell culture evaluation of shipped microcarrier beads was satisfactory - good cell growth and no contamination. The ground/flight media bags were prepared without added growth factors, and once they were degassed and determined to be satisfactory (no contamination and good color), the additional growth components (L-glutamine, chick embryo extract and antibiotics) were added (5 ml total) just before given to the STL-A technicians for the media bags' attachment to the ground and flight rails. This was done to delay component degradation that occurs at 37°C. The Cell Bead Complexes (CBC) were loaded into the cartridges and placed into the 37°C tissue culture incubator until the STL-A technicians were ready to attach them to the ground/flight rails (about a total of 1 hour in the incubator). The cartridges were attached to the rails with minimal problems and installed into the STL-A module and maintained as such until turnover and loading into the Space Shuttle *Discovery*. The only inflight activity was to monitor the control cells in the incubator for growth, contamination and fusing. Postflight processing media and reagents were also prepared. The cartridge were processed with 1-T25 flask of viable cells and 10 vials of frozen cells obtained for each L8GC (ground control) and L8SF (space flown)cartridges.

Temperature data from STS-63 indicate that the cells were maintained continuously at 37±1°C during flight. A gas leak was detected in one of the gas lines following landing. However, the color of each media bag was within acceptable range indicating that the proper pH was maintained throughout the flight. Each cartridge was continuously fed 120 ml of circulating media over the course of the flight. All cartridges were found to be free of visible microbial and fungal contamination before and after flight. Visible cell growth was observed in all of the eight "flight" cartridges (4 GC and 4 SF cartridges). Following recovery, cells from the eight cartridges were removed as per the above protocol, and placed into cryopreserving solution and frozen. An aliquot of each was also transferred to a standard tissue culture flask and maintained in continuous culture. Examination of cell yields disclosed no visible fused cells in any of the cultures at the end of flight. All eight cartridges (4 GC and 4 SF) and the L8AT stock cells (cells from ATCC cultured only in tissue culture flasks) exhibited good cell growth with no obvious contamination. All CBC from the eight cartridges were easily removed and were both frozen for shipment and plated to test for viability. Cells from all eight cartridges were viable, non-contaminated and were subsequently grown to confluency and beyond. Confluent L8AT and L8GC cells from STS-63 began to fuse on Day 4 with large pronounced regions of myotubes easily observable by Day 7. Unexpectedly, the L8SF cell cultures displayed a small number of fused cells on Day 7. By Day 11, several more fused cells in the L8SF samples were seen but no obvious

large regions of myotubes were noticed. Also, seen only in the L8SF cell cultures were regions of the previously reported (STS-45 flight) large areas of cobble-stone arrayed cells which did not fuse. The culturing of the L8 cells in the STL-A does not seem to affect the *in vitro* characteristics of L8 myoblasts (STL-A control cells (L8GC) fuse at about the same time and to the same extent as stock L8 cells (L8AT)). Exposing L8 cells cultured in the STL-A module to space flight on the Space Shuttle STS-63 mission has in some way affected either their "rate" of fusion or has abolished the ability to fuse in a subpopulation of the L8SF cells.

The cessation of cell proliferation at confluency is critical for the initiation of the normal myoblast differentiation program while the percentage of cell fusion is indicative of the degree of myoblast differentiation into myofibrils. Cells are grown in a number of 24-well tissue culture plates and labeled daily for cell proliferation (Cell Proliferation Kit, Amersham). Cessation of proliferation is indicated by a lack of labeled nuclei. Results of labeling STS-63 L8 cells a similar to those found for the STS-45 L8 cells. All STS-63 cell lines (control, ground control and flight) ceased to proliferate upon reaching confluency as indicated in a significant visible reduction in overall nuclei labeling as the cells approached confluency.

Both myosin and actin are myofibrillar structural proteins which are coordinately synthesized during differentiation and fusion of myoblasts. However, in myoblast cultures before the onset of fusion, there are low levels of myosin synthesis, which are probably related to constantly observed low level of myotubes. Synthesis of both the heavy and light chain of myosin begins just after the onset of fusion and reaches maximal levels by completion of fusion. After the initial burst of myosin synthesis, levels continue to increase until approximately 90 hrs in culture. Myosin light chain concentrations follow a similar pattern, but with a more gradual rise resulting in lower final concentrations. The link between myosin and fusion has also been clearly demonstrated in that when fusion was arrested by calcium depletion, synthesis of myosin ceased (85). Interestingly, in several non-fusing L8 clonal isolates, no increase in myosin heavy chain synthesis is observed (56). In addition, various myogenic factors have been shown to activate heavy chain myosin and alpha skeletal actin.

Cells were plated (L8AT, 2 x 105; L8GC, 1.8 x 105; L8SF, 1 x 105 cells/well) in 1 ml media onto gelatin-coated coverslips in 24 well plates and incubated at 37°C. After 1, 2, 4, 6 and 8 days, cell layers were rinsed with D-PBS and fixed with methanol. After fixation, wells were rinsed with D-PBS and rehydrated in D-PBS/BSA (0.1% w/v). The D-PBS/BSA was removed from one well at a time and 20 ul MF20* (primary antibody), which reacts with all sarcomeric myosin, or D-PBS/BSA (control) was added to the center of each coverslip. The plates were incubated for 30 min at 37°C. The coverslips were rinsed with D-PBS and D-PBS/BSA and 20 µl secondary antibody (FITC labeled goat anti-mouse) was added to each. The plates were again incubated 30 min at 37°C. All coverslips were rinsed well with D-PBS/BSA and D-PBS. Equilibration buffer from SlowFade-Light Antifade Kit (Molecular Probes, Inc., Eugene, OR) was added to each coverslip before they were mounted on slides using SlowFade mounting medium. Edges were sealed with clear fingernail polish and slides were stored at 4°C in the dark. Positively stained cells were examined under 600X magnification (oil-immersion), using a Nikon fluorescent microscope. The primary monoclonal antibody developed by Donald A. Fischman was obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, and the Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, under contract N01-HD-2-3144 from the NICHD.

All cells processed 24 hours after seeding were subconfluent. One to two L8AT cells were positively stained in each field. The positive staining pattern was primarily punctate, although some cells stained positively in a spotted pattern (where the positively stained spots were evenly distributed over the cytoplasm). Also, 3 - 4 L8GC cells were positively stained in each field. The positive staining varied from lightly spotted to complete positive staining of myosin strands. Only one positively "spotted" stained L8SF cell was seen in 3-4 fields. The "spotted" positive staining was evenly distributed throughout the cytoplasm, although lighter than that seen in the AT and GC cells. All 24 hour

negative control cells were of typical cell shape. All cells were confluent when fixed at 48 hours after seeding. Five to seven L8AT cells were positively stained per field. The shape of these cells was generally elongated with parallel sides (2 - 4 times longer than wide), although some were round to square shaped. Five to seven positively stained L8GC cells were also observed per field. These cells had not lengthened, although some did appear to be starting that process. Positively stained L8SF cells were rarely seen. The positively stained cells in all three cultures had similar staining patterns, with positively stained myosin strands generally distributed evenly throughout the cytoplasm. All 48 hour negative control cells were of typical cell shape. Four days after seeding, the L8AT culture revealed many positively stained multi-nucleated cells (many nuclei/cell) in each field with positive staining myosin strands throughout the cytoplasm. The L8GC culture looked very similar. However, among the L8SF cells, only 1-5 cells were positively stained in each field. The L8SF cells were generally elongated with parallel sides. Rare positively stained L8SF cells usually had only 2 nuclei/cell. One-two positively stained L8SF cells/field were more stellate. In all three cultures, the negatively stained control cells were very difficult to see even with phase-contrast, but the cells appeared to be of typical shape. On Day 6 after seeding, the positively stained L8AT cells were similar in appearance to Day 4, but there were more and larger tubes. The L8GC culture was again similar to the L8AT. The L8SF culture contained rare multi-nucleated (2 - 3 nuclei/cell) positively stained cells in each field similar in shape and number to those seen on Day 4. The positive staining pattern of all three cultures was similar, positively stained myosin strands running the length of and filling the cytoplasmic space. In L8SF cultures, there were also occasional spots of intense staining. On Day 8, positively stained L8AT cells remained similar in size, shape and positive staining pattern to those seen on Day 6. The monolayer of L8GC cells lifted from the coverslip and, therefore, were not processed. The positively stained L8SF cells resembled the L8AT/GC cells from Day 4 in size, general shape and staining pattern, but the L8SF cells were mononuclear. Rare multinucleated (2-3 nuclei/cell) L8SF cells were again observed. We have concluded that both L8AT and L8GC cells stained for sarcomeric myosin in the typical manner: following fusion into multinucleated cells, the sarcomeric myosin stained as long flowing fibers in cell b. A small fraction of the L8SF cells also stained brightly for sarcomeric myosin but the staining was dramatically irregular in that the cells contained small condensed "punctate" areas of stain.

The gene expression studies were initiated by assaying for the modulated expression of "known" myogenesis-related genes (MyoD, MRF4, myf- 5, myogenin and ID). Briefly, total cellular RNA is extracted from cultured control and space-flown myoblast cells at the three general stages of myoblast growth: proliferation, cessation of proliferation (upon changing to differentiation/fusion-promoting media) and fusion into myotubes. Specific gene expression screening is examined by Slot Blotting analysis and promising genes are further analyzed by Northern Hybridization analysis using appropriately labeled gene probes. Information from these experiments will verify one or more of the following space flight effects on myogenesis for each gene studied: (a) determine if space flight activates/represses specific gene expression; (b) determine if space flight enhances/reduces specific constitutive gene expression; (c) provide an estimate of the approximate size, transcription initiation site and abundance of specific space flight-regulated gene mRNAs.

Total RNA from control and space-flown myoblasts is needed to assay for the expression of "known" genes. Total RNA is isolated directly from control and space-flown cells which are subcultured once back at 1 g. RNA is isolated in the following way: Cells are lysed in a buffer with 10 U/ml of RNA Prime Inhibitor (5'--->3', Boulder, CO). The cell nuclei are then pelleted by centrifugation and the supernatant (containing RNA) is extracted by TRISOLVETM (Biotecx). The RNA is ethanol precipitated, dried briefly and stored at -85°C. Because the nuclei are removed intact, genomic DNA can also be easily isolated. The RNA concentration and purity is determined by reading a dilute sample at an OD260/280. The ratio should be approximately 1.8-2.0 for undegraded, DNA-free and protein-free RNA. Slot Blotting Analysis using appropriately labeled gene probes is carried out in order to determine if there is any altered gene expression in the space-flown cells when compared to the control L8 cells. Northern Analysis is then used to determine the amount and approximate size of specific myogenic RNA species of any genes thought to be affected by altered conditions (i.e., space flight).

An initial survey of STS-45 and STS-63 cells using the cross-hybridizing Human beta-actin (Clontech, Palo Alto, CA) indicates that beta-actin expression seems to be unaffected. We are in the process of screening the control and space-flown cells with the known muscle differentiation genes.

In summary, all our data to this point indicate that flying L8 rat myoblasts on the Space Shuttle for a duration of approximately one week at subconfluent densities leads to a permanent phenotypic and possibly a fixed genotypic change in these cells whereby significantly fewer space-flown L8 cells are able to fuse and differentiate into myotubes. Therefore, it is of benefit to: a) fly other types of myoblast cells in the STL-A under similar circumstances in the near future in order to confirm the cellular data from STS-45 and STS-63; b) detail the phenotypic/cellular characteristics which differ from L8AT and L8GC cells and which may be responsible for the L8SF deficiency of the differentiation/fusion attributes; c) delineate the molecular mechanisms of the L8SF differentiation process that may be altered by flying L8 myoblasts at microgravity. Specifically, are certain expressed genes more susceptible to space flight while other sequences simultaneously remain refractory to microgravity regulation? Are certain types of cells more susceptible to space flight (i.e., pluripotent vs differentiated) and is the stage of cell cycle/fetal development important (on STS-29 all sixteen 2-day old chick embryos died, having stopped development) during the flight? Once the specific space flight-regulated mechanisms are determined, the exact time sequence of effects can be established. Results will also significantly expand our knowledge regarding cellular response to microgravity, while also having important implications for further space flight with respect to muscle injury, exercise, surgery, cancer and fetal development.

Manned space missions have increased in duration from 108 minutes to 366 days during the last 30 years. Orbital space stations now make it possible to perform extensive biological and medical research in space. One of the areas receiving considerable research attention is the effect of long-duration space flight, specifically microgravity, on the musculoskeleton system because it is commonly recognized that microgravity is the major limiting factor in the body's adaptation to reduced environmental requirements. The musculoskeleton system's processes of a) wound healing following trauma or surgery; b) bone healing following fractures; and c) muscle atrophy regeneration, as well as general muscle cell proliferation and differentiation, are all thought to be dramatically altered under the influence of microgravity. Therefore, the specific goal of our extended myoblast cell culture model proposed in this protocol is both to continue augmenting previous whole animal model studies in these areas and to help simplify the molecular and cellular analysis of microgravity effects on wound healing and muscle atrophy regeneration, as well as on muscle cell proliferation and differentiation in general. The proposed extended research will be conducted with the following aims in mind: A) Reinforce the role of microgravity in regulating the proliferation and differentiation programs of various skeletal muscle myoblast cell lines by corroborating the cellular results of previously space-flown L8 myoblast cells. Of particular interest is information as to whether the lack of gravity affects the *in vitro* cellular proliferation and differentiation programs of several other well studied myoblast cell lines (non-tumorigenic mouse C2C12 and G-8 and rat L6 skeletal myoblasts). Specifically, are the low- or non-fusing variants specific only to the L8 cell line or can these changes be induced in other well studied myoblast cell lines? In addition, is the cell proliferation program of other myoblast cell lines directly affected or is the cessation of cell proliferation merely a response to decreased growth factors? Specifically, which mechanisms involved in cell fusion/differentiation are modulated by microgravity? And finally, does exposure to microgravity initiate events leading to permanent phenotypic/genetic changes or can the return to 1-g reestablish the normal myoblast growth and differentiation program? B) Determine whether any of the observed phenotypic changes are a direct result of microgravity-modulated gene expression. Does microgravity affect "known" genes? Specifically, are microgravity-regulated genes directly related to "known" myoblast proliferation- or myoblast fusion/differentiation-regulated genes (i.e., MyoD, myogenin, MYF-4, etc.)? Are the mechanisms of microgravity-induced changes similar in all myoblast cell lines and can they possibly be present in other differentiating cell types? C) Ultimately, analyze the common mechanism(s) by which microgravity may affect the genetic expression pattern of cultured myoblasts specifically, and other cells in general. Are these

microgravitational adaptation mechanisms specific to differentiating cells or are there general microgravity-response mechanisms found in other cells?

While the specific scientific aims of this program are directed towards increasing our understanding of the relationship between the actions of microgravity and cellular functions, they also may contribute information of a broader biological relevance: A) Identify those genes whose function may be involved in normal/abnormal muscle wound healing and muscle atrophy regeneration. This is a plausible prospect in view of recent studies concerning the involvement of muscle adult myoblasts (satellite cells) in the mechanism of wound healing. How is wound healing affected at the cellular and molecular levels by the microgravity environment of space? Can any of the problems ascribed to wound healing in space be compared to problem wound healing here on Earth? In addition, what are the changes (if any) in the role of the satellite cells in muscle atrophy regeneration following exposure to the microgravity environment? B) Provide a rationale for the clinical application of intervention therapies in the treatment of microgravity-induced physiological alterations. Data may be used to develop pharmaceutical products and more effective physical treatment regimens to limit the extent of muscle tissue loss and accelerate possible wound healing resulting from exposure to extended periods in the microgravity environment. Anticipated benefits include savings from reduced need for physical therapy, enhanced flight team cohesion and a more rapid return of personnel to duty status following injury. Significant negative impact on flight crew performance upon return to the 1-g environment is suggested and the feasibility of interplanetary missions may hinge on the development of effective measures to prevent or limit tissue loss.

A multitude of physiological changes occurs in humans exposed to various periods of weightlessness in space. To date, these changes have not resulted in obvious disease or impairment of performance in flight. The extent to which physiological alterations will progress as periods of weightlessness increase is unknown. Upon return to gravity, these changes may impair the ability to function properly. In addition, a practical method of inducing gravity in a space station (i.e., rotation) presents enormous challenges. Therefore, further study of cellular and molecular changes and measures to counteract the effects of microgravity are essential with the hope that findings will allow scientists to better understand and combat these problems on Earth.

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An Experiment to Study the Role of Gravity in the Development of the Optic Nerve

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Funding:

Project Identification: RF-65

Solicitation: 93-OLMSA-03

Initial Funding Date: 5/94

Expiration: 5/96

FY 1995 Funding: $203,489

Students Funded Under Research: 3

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: Ames Research Center

Task Description:

The purpose of this experiment is to identify changes in development of the optic nerve in rats exposed to the weightlessness of space prior to birth. The results of this research may help us begin to understand the way in which gravity influences the development of our visual system. In young animals, the final route or destination of electrical impulses from eye to brain is not well defined. The brain receives "fuzzy images" of the world because images captured by the eye may be sent to a range of points in the brain's visual center. The microgravity environment of space may indirectly play a role in modifying the retina to brain signal pattern.

The accuracy of optic nerve connections between the retina and the brain was studied in ten rats which underwent gestation in space and compared to the accuracy of those connections in ten rats which underwent gestation on Earth. Two day old rat pups had very inaccurate connections in both groups though there appeared to be more variability in those pups from the space flight group. By 14 days of age, the accuracy of optic nerve connections was at the normal adult level for both groups.

It appears that gestation in space flight does not significantly affect the inherent early inaccuracy of optic nerve connections in rats, nor does it affect the subsequent postnatal maturation to accurate connections. It remains to be seen whether the postnatal maturation of these connections would be affected by continued postnatal rearing in space.

Since neuronal connections between the eye and the brain appear to develop normally in weightlessness, it is not likely that gravity plays a significant role in the development of neuronal

connections in general. Animals or humans reared in space could be expected to have normal vision. Inaccurate neuronal connections due to weightlessness might be expected to be confined to those parts of the brain concerned with balance or motion sensitivity.

Although the major factors responsible for determining the accuracy of optic nerve connections remain undetermined, it appears that researchers concerned with developing techniques for optic nerve regeneration as a treatment for blindness need not worry about the role that gravity may play in developing proper optic nerve connections.

Publications, Presentations, and Other Accomplishments:

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Influence of Space Flight on Bone Cell Cultures

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Funding:

Project Identification:

Solicitation: 93 OLMSA-04

Initial Funding Date: 1/95

Expiration: 12/95

FY 1995 Funding: $102,173

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-C3 (STS-63, 2/95)

Responsible NASA Center: Ames Research Center

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. 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.

Cultures of osteoblasts obtained from normal 17 day old embryonic chick bone (*calvaria*) were flown on the STL module during NASA shuttle mission STS-63 and compared to the same cells maintained under normal gravity (1g). Measurements have been made or are being completed on the metabolism of the bone cells and aspects of their molecular biological, biochemical, and structural nature to determine possible effects of space flight and microgravity on the cultures.

To date, examination of the structure of the cultures by electron microscopy has shown that the flight cells grow and develop in space flight and produce considerable amounts of an extracellular network

composed principally of collagen. These observations are similar to those found for the counterpart control cells (grown at 1g for the duration of the launch and flight and terminated on retrieval of the flight samples) and basal cells (grown at 1g and terminated on the day of launch). Specific differences between the 3 groups of cells in measurable quantities or the nature of collagen or other extracellular proteins have not yet been determined.

Molecular biological and biochemical analyses of the cultures are being made in terms of the gene expression of type I collagen, osteopontin, osteocalcin, and fibronectin, all proteins important in bone formation and mineralization. At the present time, comparisons of the 3 cell groups have shown detectable message levels of all four of the proteins of interest, and some differences have been detected in the presence of the respective RNA expression. Interpretation of the changes is premature at this time although there are indications that the expression of osteopontin and fibronectin, proteins containing the common cell-binding RGD sequence, is enhanced in space flight, possibly to maintain a spread configuration of the cells while compensating for the weightless environment.

The current metabolic studies of glucose utilization and lactate production have shown that all 3 cell groups had consumed available glucose equally rapidly; however, basal cultures produced greater lactate amounts compared to both flight and control cultures. Since basal cells were fed continuously to the time of launch while flight and control cells were first fed continuously until 2 days before launch and then placed on an intermittent feeding schedule in STL modules, the lactate differences would indicate that the maintenance levels of feeding for the flight and control cells in their respective STL units may have been inadequate. The result is that these cultures may have been retarded in their ability to grow as completely as possible over the duration of the launch and flight. Additional studies of the bone cells placed and isolated in the STL module at 1G would be necessary to confirm this result. In the possibility that the cultures may be only slowly growing and developing, the data collected and analyzed regarding gene expression and cell matrix structures are likely to reflect smaller levels than might otherwise be expected. Further studies into all aspects of analyses are continuing.

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.

Publications, Presentations, and Other Accomplishments:

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Osteoblast Adhesion and Phenotype in Microgravity

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Project Identification:

Solicitation: 93 OLMSA-04

Initial Funding Date: 1/95

Expiration: 12/95

FY 1995 Funding: $13,248

Students Funded Under Research: 0

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-C6 (STS-80, 11/96)

Responsible NASA Center: Ames Research Center

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 middeck compartment of the space shuttle in the Space Tissue Loss (STL) culture device during a planned 11 day flight. Parallel control cultures will be maintained on Earth under identical conditions. During the 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 on this project during FY95 were directed primarily at preparations for flight STS-69. These included training and experience with the STL module and facilities at Kennedy Space Center. The flight STS-69 took place between September 7 and September 19, 1995. Samples were successfully

recovered from the flight and were transferred to Mount Sinai Medical Center for analysis. These studies have been initiated but results are not yet available for 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.

Ca^{2+} Metabolism and Vascular Function After Space Flight

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Solicitation: AO-93-OLMSA-01

Initial Funding Date: 7/95

Expiration: 12/95

FY 1995 Funding: $150,000

Students Funded Under Research: 0

Flight Information:

Flight Assignment: PARE-04 (STS-85, 1997)

Responsible NASA Center: Ames Research Center

Task Description:

This research application proposes to explore the consequences of space flight on calcium metabolism and cardiovascular function. During the past 15 years, an extensive body of research literature has characterized a consistent and theoretically plausible link between alterations in calcium metabolism and dysregulation of cardiovascular physiology. The perturbations of calcium metabolism include decreased intestinal absorption of calcium and reduced renal reabsorption of calcium, failure of subcellular calcium regulation, and abnormal expression of the ubiquitous cellular calcium binding protein calmodulin. These organ and subcellular disturbances result in a variety of abnormalities of organ and cellular function including hypercalciuria, hypocalcemia, hypophosphatemia, suppressed 1,25(OH)2D3 levels, increased parathyroid gland mass and circulating parathyroid hormone (PTH) levels, and reduced bone mineral mass. All of these have been associated with increased arterial pressure and vascular dysfunction.

Given the links between calcium metabolism and cardiovascular function and the profound changes in calcium metabolism that occur during space flight, it follows that space flight could alter cardiovascular function as a consequence of altered calcium metabolism. We propose to explore these possibilities by assessing acute changes in vascular function as well as systemic and cellular calcium metabolism following space flight in an animal model of experimental hypertension that is known to have deficits in calcium metabolism.

Dietary calcium will be manipulated to determine the effectiveness of diet as a countermeasure for the consequences of zero gravity. Animals will be pretreated with high or low levels of dietary calcium from weaning until space flight at seven weeks of age. It is anticipated that high levels of dietary

calcium will ameliorate the impact of weightlessness on calcium metabolism. The findings of these experiments will provide evidence as to whether additional studies in animal models and in human subjects should be undertaken to further characterize consequences of the effects of zero gravity on calcium homeostasis and subsequent vascular dysfunction. Furthermore, these results will provide information as to the effectiveness of pre- and post-flight manipulations of dietary calcium on subsequent systemic and cellular calcium metabolism and cardiovascular function.

The overall goal of the Physiological and Anatomical Rodent Experiment (PARE-04) is to evaluate the relationship between dietary calcium, calcium metabolism, blood pressure, and vascular function in zero gravity conditions. Much of the detailed methodology for this project has been established through previous research. However, two important questions remain to be answered. One relates to the precise formulation of the experimental diet that the rats will be fed. NASA currently uses a dietary formula that can be extruded into spacebars. It is not known how variations in dietary calcium will interact with the nutrients contained in that diet. Nutrient interactions could influence absorption and metabolism of calcium and therefore could modify the effect of dietary calcium on the cardiovascular function. The other question concerns the strain of rat to be used. The bulk of our previous research on dietary calcium and blood pressure has been done with spontaneously hypertensive rats obtained from Charles River Breeding Labs. In contrast, NASA has used Taconic Farms as their primary animal supplier because of their demonstrated ability to meet the demanding schedule required in flight research. Unfortunately, rats from Charles River do not respond to dietary electrolytes in the same way as rats from Taconic Farms.

To provide answers to these questions, we conducted a ground study using four levels of dietary calcium and two strains of rats. The experimental phase of the ground study was completed in late December. Analysis of the data is ongoing. Preliminary results from the study indicate that optimal contrasts will be obtained with 0.2% calcium as the low calcium diet and 2.0% calcium for the high calcium diet. These two diets were found to result in significantly different blood pressure levels in both Taconic Farms and Charles River rats. Further, platelet intracellular calcium levels differed significantly across diets in both strains. We are currently interfacing the data from the vessels with that of blood pressure and platelet intracellular calcium to determine whether the diets had a consistent effect on the vasculature in both strains of rats. When that analysis is completed, a recommendation can be made regarding the strain of rat to use in the flight experiment.

There were several additional benefits from the ground study. It provided a hands-on opportunity for the team to go through the protocol and experience the practical problems involved in coordinating several aspects of the experiment. This was extremely useful and will pay dividends in the success of the flight experiment. The ground study also afforded an opportunity to hone the detailed methodologies including drug dosages and optimal incubation times. Likewise, we were able to test a new fluorimeter system and establish its reliability. As a result of the ground study we are in a much better position to successfully complete the flight study.

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 effects 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 Earthbound.

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Microgravity Effects on Early Reproductive Development in Plants

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Funding:

Project Identification:

Solicitation:

Initial Funding Date: 11/94

Expiration: 11/95

FY 1995 Funding: $141,384

Students Funded Under Research: 7

Flight Information:

Flight Assignment: STS-68

Responsible NASA Center: Kennedy Space Center

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 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 the 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.

Analysis of reproductive material from Chromex-04 and Chromex-5 has been completed. The results were presented at the annual meetings of the American Society for Gravitational and Space Biology and American Society of Plant Physiologists. Two manuscripts detailing reproductive development in microgravity are in press (*Planta*) or in review (*Annals of Botany*), and our report on early reproductive development in *Arabidopsis* during Chromex-03 was published in the *American Journal of Botany*. The results were also presented by the PI at numerous invited seminars across the country. In addition to the primary task of analyzing the reproductive material from these studies, we have to understand the

physiological activity of the plants during space flight by examining root and shoot parameters. Specifically, activity of the enzyme alcohol dehydrogenase was examined in the roots, and concentrations of starch and soluble carbohydrates was assayed in the foliage. Work continues on manuscript preparation on these topics. An additional contribution was the completion of a baseline study on pollen development in *Arabidopsis*. A manuscript from this study is currently in review with Protoplasma. Work is now underway to prepare for the new flight opportunity (CUE).

Considering the importance of seed production in the plant's life cycle, it is unfortunate that we have little information on the fate of this complex developmental process in microgravity. Indications from the Soviet experiments all point to a very interesting and important developmental interaction of events during plant reproduction and microgravity. These flight experiments will pinpoint the developmental events which are disturbed in microgravity and further will give indications as to whether this is a direct effect of microgravity on a developmental process or whether physiological events set in motion by secondary effects of microgravity on the plant microenvironment might be contributing to the developmental lesions. While the current Shuttle flights are not of long enough duration to complete a full plant life cycle in space (even with our selection of plants with a very short life cycle), the experiments are able to pinpoint developmentally sensitive times in the early reproductive processes. These experiments lay the groundwork for a true seed-to-seed cycle experiment which we would propose for longer term flight opportunities on Mir or the International Space Station. These experiments give space biologists a chance to look at what developmental events beyond the seedling stage of a plant are affected by microgravity. Furthermore, the information gained on microgravity effects on seed set will be of utility for Space Life Scientists who intend eventually to assist human habitation of space with a plant-based food supply. Such basic research on the important processes of plant reproductive development will strengthen our knowledge base and lead to improvements in crop productivity on Earth.

Effect of Microgravity on Bone Development

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Funding:

Project Identification:

Solicitation: 93-OLMSA-03

Initial Funding Date: 1/95

Expiration: 12/96

FY 1995 Funding: $127,979

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

Responsible NASA Center: Ames Research Center

Task Description:

Dr. Partridge 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.

Our flight with our experiment occurred in July, 1995. Everything went extremely well. I was very happy that we managed to obtain a 9 day flight and that landing was at KSC. The dissections went very smoothly from mid-July to early September with no problems except for Hurricane Erin moving the PN7 dissection to PN8. The samples were shipped to St. Louis uneventfully, with all arriving as requested. We then started the analysis of the calvarial samples, cutting sections and doing immunohistochemistry for collagenase and tissue plasminogen activator. We will have these completed by the end of FY1996. We have also been in contact with Dr. Sue Bodine about the possibility of obtaining some of the pup tibiae for analyses. The only materials which we have not received and which we requested are the details of the pup handling and the videos of the dams immediately after landing. Overall, I am sure that we will be able to achieve the scientific goals we have proposed.

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.

Publications, Presentations, and Other Accomplishments:

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Microgravity and Placental Development

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Funding:

Project Identification: 5-01131

Solicitation: 93-OLMSA-03

Initial Funding Date: 5/94

Expiration: 12/95

FY 1995 Funding: $81,382

Students Funded Under Research: 0

Joint Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: Ames Research Center

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 have 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.

Morphological and biochemical aspects of the task have been completed. Analysis of hormone expression is partially completed. Results indicate that space flight of pregnant animals beginning after embryo implantation does not affect placental structure and selected biochemical parameters. Hormonal analysis will be completed as proposed. An important question generated by this study is whether space flight would influence embryo implantation which is a critical event governing reproductive success.

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 study 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.

Publications, Presentations, and Other Accomplishments:

Renegar, R.H. "Morphological and Functional Parameters of Placentas from Rat Dams Flown on the NIH.R1 Study." Platform Presentation, Annual Meeting of the American Society for Gravitational and Space Biology, 1995.

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Flight Verification Test of Nursing Facility

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Funding:

Project Identification:

Initial Funding Date: 8/94

FY 1995 Funding: $75,000

Joint Participation: NIH

Solicitation: AO-93-OLMSA-01

Expiration: 1/97

Students Funded Under Research: 3

Flight Information:

Flight Assignment: NIH-R3 (STS-72, 11/95)

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 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 for comparison. 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.

Analysis of flight and ground control data was begun February 1996. Preliminary assessment indicates that the NF cage is biocompatible for neonatal rats 8 days and older which survived space flight in good to very good health compared to vivarium-housed and NF-housed ground controls. Thus, 8-day-old and older neonates appear suitable for Neurolab studies. The present NF cage design was not biocompatible for space flight of neonates 5 days old because only 30% of the flight PN5 neonates survived compared to 100% survival of ground controls. Body weight gains of PN8 flight animals were lower than ground controls suggesting that modifications of the NF cage are advisable for improving litter huddling and nursing in microgravity. Further examination of data will result in recommendations for improving the configuration of the NF flight cage.

Analysis of video, body weight, muscle weight, and histology data will continue to obtain a more accurate assessment of neonate and dam biocompatibility with the NF cage and the influence of microgravity on neuromuscular development. Surviving flight and ground control animals will be processed 2-3 months postflight to determine whether long-term changes were induced.

This successful mission represents a milestone demonstrating that immature mammals can develop in space. This is an important first step to raising animals on the International Space Station for research, and the less immediate scenario of humans being born and developing in space.

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 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.

Microgravity Effects during Fertilization, Cell Division, Development, and Calcium Metabolism in Sea Urchins

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Funding:

Project Identification: A144 BF78 A48 9700

Solicitation: 95-OLMSA-01

Initial Funding Date: 3/92

Expiration: 2/95

FY 1995 Funding: $109,922

Students Funded Under Research: 2

Flight Information:

Flight Assignment: ARF-1 (STS-77, 1996)

Responsible NASA Center: Kennedy Space Center

Task Description:

During FY 95, there were no changes from the original proposal and the research conducted followed that described in the original proposal. Specifically, investigations continued to condition ground-based experiments for in-flight investigations on the space shuttle orbiter in May of 1996.

The rationale behind these experiments is that gravity has already been shown to affect bone calcium, and it may well influence processes during fertilization, cell division, development and embryogenesis. This project is exploring the role of microgravity during fertilization, early development, cytoskeletal organization, and skeletal calcium deposition in a model developmental system: the sea urchin. In doing so, we have also helped CSA develop, test and fly the aquatic research facility (ARF) system. During FY 95 this system has been tested successfully at the Kennedy Space center in September of 1995 and we could demonstrate that development of sea urchins occurs normally in the ARF hardware system. 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 was investigated by light microscopy, and 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). The model system employed for this study is the sea urchin, which will deposit extracellular calcium to form spicules within two days of insemination. These light-weight experiments on animals grown at near-room temperatures hold promise for generating a full appreciation of the role of gravity in fertilization, embryonic axis determination, cell division, cytoskeletal organization, and the effects of microgravity on bone calcium deposition.

During the past year this group has made very significant progress in determining the feasibility of flying an experiment to test the effects of microgravity using sea urchin eggs as a model system. Sea urchins have now been flown aboard a KC-135 and fertilization rates equivalent to those on Earth have been obtained in the first 24 seconds of weightlessness although sperm movement was somewhat slower. During this past year, in collaboration with CSA, chambers have been developed which allow for sea urchin development to the pluteus state. Additionally, methods have been developed to simultaneously analyze cells with immunofluorescence and electron microscopy which are detailed in the paper published in AIAA-95-1059.

The methods developed for this project have been applied to one-cell, 2-cell, 8-cell, 16-cell embryos, as well as 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 x-ray microanalysis to characterize the calcium deposits during late gastrula and pluteus stages. After successful ground-based experimentation, these methods will be used on cells cultured under microgravity conditions on the space shuttle planned for May 1996.

Major efforts during the past year were devoted to identifying the most suitable biologically compatible material and hardware to determine the optimal conditions for the conduct of the experiments on the space shuttle. Successful collaborations with MOB Technologies and the Canadian Space Agency have led to tests determining the compatibility of constructed materials with sea urchin gametes, ability to maintain life-support parameter proper gamete ratios and volumes to attain fertilization, proper fixation ratios, volumes and composition to preserve specimens for post-flight analysis, and reliability of dilution and mixing in the hardware components. After testing several prototypes of hardware components and f fixatives, the designs we arrived at are described in more detail in the manuscript entitled: "Modification of experimental protocols for a space shuttle flight and applications for the analysis of cytoskeletal structures during fertilization, cell division, and development in sea urchin embryos" which has been published by the American Institute of Aeronautics and Astronautics. This work was presented at the Life Sciences and Space Medicine Conference held in Houston from April 3-5, 1995.

A successful execution of a Science Verification Test has been conducted at the Kennedy Space Center, in conjunction with the Canadian Space Agency and MPB Technologies. Component hardware designs have been tested for supporting sea urchin In-flight fertilization and development for a future flight on the mid-deck locker of the orbiter spacecraft. The development of new fixation protocols has allowed us to preserve delicate cytoskeletal structures, cell organelles, and calcium-sequesting systems for post-flight analysis, which has proven very successful during the Pre-Science and subsequent Science Verification Test at the Kennedy Space Center

1. The compatibility of all hardware components with gamete viability has been confirmed. Specifically, the materials used have been found to be non-toxic, and semi-permeable to allow oxygen/carbon dioxide exchange.

2. Maintenance of the appropriate parameters in flight including temperature (12°C), g-force in the quasi-stationary centrifuge, g-force in the control 1-g centrifuge, and the archival of the time diluted sperm is added to the unfertilized eggs as well as the time of fixative addition at preselected time points has been verified. Mock post-light environment parameters including pH, salinity, and oxygen levels have also been examined at the end of simulated testing of hardware modules.

3. Gamete ratios and volume of sperm to eggs and sperm/eggs to seawater within the specified specimen enclosures have been determined. This includes appropriate injection volumes, flow rates, and concentrations of sperm for the insemination subsystems as well as fluid mixing.

Specifically, the ARF unit has been tested to verify that it will: 1) initiate insemination within 3O hours of egg spawning; 2) keep the eggs, sperm, and fixatives separate until the proper time required and confirmation that the correct sequence of fertilization followed by fixation is maintained; 3) predilution of "dry", concentrated sperm by gentle mixing without damaging sperm cells; 4) introduction of sperm suspension within 1 minute of initial seawater contact; and 5) the immediate and gentle mechanical mixing of sperm/egg solution for a 3 minute duration.

4. Appropriate fixatives, concentrations, and volumes have been studied with respect to seawater volumes and total volume constraints of the ARF specimen enclosures and fixation subsystem. Specifically, the fixation subsystem has been tested to: 1) flax duplicate sets of eggs in both l-g and O-g flight centrifuges; 2) fix at the following critical time points, subject to verification of the timing of fertilization and development of sea urchin eggs in the ARF specimen unit (all times given post-sperm addition): 3 minutes for sperm/egg fusion and cortical granule reaction; 20 minutes for pronuclear migration and fusion; 9O minutes for metaphase of first mitosis; 12O minutes for cytokinesis; 4 hours for the determinative 4th cleavage; 48 hours for gastrulation; and 72 hours for specule formation in plutei larval stage; and 3) support gentle agitation of the gamete-fixative solution to ensure good specimen preservation for post-flight analysis.

A number of questions have been answered during this past FY95 and it was demonstrated that sea urchin embryos can be grown up to the important plateaus stage which is a major improvement over previous culture conditions. Consistent analysis and changes of several parameters in the culture medium led us to arrive at the favorable conditions described in the appended manuscript. Additionally, a breakthrough was achieved with the successful determination of fixation protocols which allows us now to reliably analyze the structures of interest with immunofluorescence and electron microscopy simultaneously. The conditions for the analysis of early and late stages of development were determined, and it was concluded that a higher concentration of eggs and sperm could be used for the culture of early stages, but a lower concentration was necessary for optimal culture of embryos to later stages. Also, different fixation protocols needed to be applied to obtain optimal results for the different stages. These protocols have also been employed to test the effects of clinostat rotations on tissue culture cells and the major finding in these studies was that under these conditions of simulated microgravity, centrosome organization is affected. This finding will be important for the interpretation of results obtained for the sea urchin system as centrosomes are the structures which organize microtubules and define polarity and cell differentiation. If centrosomes are affected during development in the microgravity environment, we can expect a certain percentage of embryos to develop abnormally or disintegrate. If centrosome organization were to be affected under microgravity conditions, birth defects could be the result during later development. The analysis of centrosomes will also have implications for cell division. If centrosome regulation is affected by microgravity, cell division will be abnormal and the risk for cancer may be increased under microgravity conditions.

During the analysis of centrosomes, interest was generated for the function of centrioles which are microtubule-based organelles that typically are encapsulated in centrosomes in most cells except for plant and some animal cells. As of today, the function of centrioles remains obscure and it was hypothesized that they may be organelles sensing the direction for cell movement . In terms of evolution, centrioles may have been the key organelles during development to adapt to a gravity environment, and may have served similar functions on a cellular level as otoliths on a physiological level When adaptation was completed, these organelles may have lost their function. If the hypothesis is correct that they may be sensors for cell movement and direction of cell growth, then their ultrastructural organization may be altered in microgravity.

Aside from being inclusions in centrosomes in most cells except for plant cells and some animal cells, specialized forms of centrioles are found in form of basal bodies in cilia and flagella. During sea urchin development, cilia beating allows for free swimming of the blastula and gastrulae embryos. It will be interesting to analyze if swimming behavior is altered in the microgravity environment and if the

ultrastructure of basal bodies is altered due to modified cilia function. In preliminary tests on the KC-135, it was observed that swimming of sperm is slowed down as compared to control sperm.

With the successful development of the AIF hardware system and with successfully defining the growth conditions for culturing sea urchin embryos up to the pluteus stages in this temperature-controlled culturing system, the analysis of later stages of development has now become enormously interesting. As outlined in the previous paragraph, cilia movement is of great interest to answer questions concerning microorganisms that rely on cilia for cell movement, for a diversity of aquatic animals that use cilia for locomotion, and most of all, for biomedical research. Cilia serve numerous functions in the human body. They are needed in the ovaries to move the oocytes, and they serve very important functions in the auditory, the olfactory, and the respiratory system. The experiments conducted on the space shuttle in May of 1996 will give us some insights into the questions if cilia are affected by microgravity but this aspect of the present research will need to be analyzed in more specific detailed experiments in the future.

Similarly, the analysis of spicule formation in the pluteus embryo will be of great biomedical value as spicule formation is the result of deposition of calcium carbonate on an organic matrix which will prevail us with insights into the calcification of bone tissue in the microgravity environment Having worked out the parameters to successfully grow plutei under controlled conditions in the ARF system, we now can ask specific questions about this calcification process by adding or deleting certain factors in the growth medium during the exposure to microgravity. With more sophisticated forms of microscopy such as x-ray diffraction we then can analyze the exact composition of deposited material in the calcified organic matter. These experiments will contribute to research on osteoporosis and loss of calcium during space flights. The loss of calcium in astronauts (up to 460 mg/day) extrapolates to 25% of the total body calcium in a year of space, effectively precluding long-term missions. Base research on this phenomenon can be addressed in the sea urchin system during embryo development. The utility and speed of this example of the formation of an extracellular skeletal array will serve as a significant model in which to evaluate the effects of microgravity on calcium deposition.

We are confident that our progress made during this year, will affect future work on this task positively By extrapolating our present techniques to the conditions on the Shuttle Orbiter, we will further the understanding of basic events during fertilization, cell division and development, as well as answer critical questions regarding the space biology of cytoskeletal and skeletal organization.

Several aspects of this research are aimed at understanding diseases that affect humans on Earth. Since the cytoskeleton is most important for many processes in the cell including signal transduction, hormone secretion, organelle transport, cell shape changes, fertilization, cell division, cell polarity, and many more, these studies will provide an important foundation for the studies on osteoporosis, neurological diseases, aging, and reproduction. Benefits from studies exploring cytoskeletal organization and calcium metabolism in this system will come in form of generating database information and identifying target sites for pharmaceutica which would interfere with pathological conditions found in osteoporosis and other diseases related to the cytoskeleton such as reproduction, cancer, artheriosclerosis, aging, and a variety of neurological diseases including Alzheimer ' s.

Birth defects can have various causes and our studies are focused on investigating the cytoskeletal components that play a crucial role during the development of a healthy embryo. Environmental factors can cause cellular structure to malfunction and by studying the basic processes during development we will investigate if microgravity has an effect on the cytoskeletal system and calcium metabolism in the developing embryo. These studies will benefit research in reproduction in space as well as on Earth.

Fertilization and cell division include also processes which relate to fundamentally and universally important key events found in the nervous system and during muscle movement. The sperm aster during fertilization and the mitotic apparatus during cell division are microtubule-based structures that

are contractile and serve similar functions in a cell as muscles do in the human body. By studying the contractile structures during fertilization and cell division in a simple model system we can extrapolate to cell functions in more complex systems that might be affected by microgravity. These studies will contribute to diseases of the muscle, bone, and nerve system on Earth.

The cytoskeleton consists mainly of small structures that are microfilaments, intermediate filaments, microtubules, and in most cases, their organizing centers, the centrosomes. The interactions and proper balance of these structures is the basis for proper cellular functions. Any biochemical imbalances of these structures can lead to disease of muscle and nerve cells as seen in muscular dystrophy or Alzheimer's. The onset of Alzheimer's is accompanied by imbalances in phosphorylation of the microtubule-associated protein complex. This imbalance results in a decrease of microtubule function and an increase of non-functioning intermediate filaments that can be observed with electron microscopy as paired helical filaments in the diseasing brain. In the present studies, we will determine if microgravity will affect the balance between microfilaments, microtubules, and intermediate filaments.

Microtubules also play a major role in secretion as they are the structures transporting cellular organelles. By doing so they provide the specific ionic and calcium requirements for specific events in cells and tissue. Secretion is a major event during innumerous cellular processes and there are indications from previous studies that secretion might be affected by microgravity which would have many implications on cells in our body. Cortical granule exocytosis triggered by the fertilizing sperm has been viewed as a model system to study secretion. These processes have direct similarities to synaptic vesicle secretion in nerve cells.

Proper cytoskeletal functions are based on proper calcium sequestration. If there are imbalances in calcium metabolism, the cytoskeletal system will not function normally and diseases may have their onset at very early stages of development that may result in muscle or bone diseases later on. Throughout development, many processes in the developing embryo are driven by calcium, and aside from studying the early calcium events, we will also investigate if calcification of spicules is affected by microgravity. This research will benefit the studies on osteoporosis. Astronauts traveling in space experience similar losses of calcium as humans on Earth who are aging or are not using their muscles. Osteoporosis can be detected in form of structural differences in the human bone. By using a simple system such as the sea urchin, we can investigate if calcification is affected by microgravity in similar ways as during muscle and bone diseases on earth. If structural differences are found, these studies will help research on osteoporosis in the human body as spicule formation in sea urchin Pluto can be compared in a number of ways to bone formation in the human body.

The other major and novel structures that play a crucial role during cell division at all stages of development are centrosomes. Centrosomes are the most important structures that organize microtubules, and some cases of infertility are based on malfunctioning centrosomes during fertilization. If centrosomes are malfunctioning, microtubules can not be organized and the union of paternal and maternal genomes does not take place. on the other hand, centrosome impairment during cell division can result in failure of cell division which would affect processes such as would healing Uncontrolled centrosome function could lead to uncontrolled cell division such as in cancer cells . There are indications that cell division is impaired in some cell types when subjected to microgravity, and it is likely that the reasons for that are based on impaired centrosome function during cell division. Effects on centrosomes could increase the risk

This project is also focused on the effects of microgravity on cilia. Cilia are important for many functions in our body and the investigations can prove valuable for the interpretation of cilia-related functions and impairment during future space flights and for diseases on Earth. For example, cilia in the ovary are responsible for moving the oocytes. Cilia in sperm allow for swimming and locomote the male DNA into the egg. Cilia are also very important in the auditory system to allow hearing, and in the olfactory system to allow smelling. Celia are oftentimes also associated with microfilaments

that are found predominantly in microvilli at the cell surface, in the microvilli of intestines, and in the auditory and olfactory system. It has been shown beautifully that microvilli in the auditory system are irreversibly destroyed when they are subjected to loud noise. Studies on cilia and microvilli in the sea urchin under microgravity conditions may prove valuable to extrapolate on cilia and microvilli found in the human body.

The studies in this project also investigate calcium metabolism. The loss of calcium in astronauts during space travel extrapolates to 25% of the total body calcium in a year of space, effectively precluding long-term missions. The experiments performed in this project are aimed at investigating the mechanisms involved in calcium loss which eventually might help to determine the sites for metabolic calcium imbalance and aid in the design of pharmaceutica that help prevent osteoporosis. This aspect of the research has fundamental implications in bone research with particular focus on osteoporosis.

The experiments conducted in this project are investigating basic cellular functions. By determining the sites for malfunction, strategies can be developed that identify target sites for pharmaceutica that could correct the affected areas. Since the studies address a variety of basic cellular questions, these pharmaceutica could be directed to correct diseases such as Alzheimer's, muscular dystrophy, osteoporosis, and cancer.

A variety of basic biological processes are being explored in these studies. Among these are the process of secretion during cortical granule exocytosis that has direct parallels to secretion in a number cells such as nerve cells, pancreas cells, and gland cells . The sea urchin system has served as a classic model system to study these fundamental processes.

Understanding the cytoskeletal system and the centrosome will lead to understanding major processes in the human body. Centrosomes, and centrioles in particular, are very poorly investigated although they play major roles in processes where microtubule function is required. The studies conducted in this project are expected to contribute greatly to the understanding of the involvement of 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 muscle. Research on the cytoskeleton and skeletal formation will benefit processes on Earth and in space as they relate to secretion, cancer, neurological diseases, muscle diseases and birth defects.

The design of pharmaceutica that might follow the discovery of sites that are affected in diseases where cytoskeletal structures play a role could directly benefit the common man affected by diseases such as osteoporosis, cancer, and neurological diseases including Alzheimer's.

The development of the ARF system will benefit all future investigations employing this system for biomedical or aquatic research .

The development of novel cytological protocols will benefit a variety of investigators in cell, developmental, and neurological research the experiments we have conducted on ground proof that the ARF system can now he utilized to perform experiments in space requiring controlled temperature, humidity, illumination, as well as in-flight fertilization and fixation at predetermined time points. Controlled experimentation is a prerequisite for obtaining solid science results. For the first time, such controlled conditions will be available and employee for the experiments conducted to investigate development of ~ representative aquatic animal system that represents in many aspects events during mammalian fertilization more accurately than those studied in the typical mammalian models of mice and hamsters.

The development of fixation protocols during this project to preserve delicate cytoskeletal structures with immunofluorescence and electron microscopy will benefit a great number of researchers in the fields of cell biology, developmental biology, embryology, and neurology.

Publications, Presentations, and Other Accomplishments:

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Effect of Spaceflight on Development of Immune Responses

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Funding:

Project Identification:

Solicitation: 93-OLMSA-03

Initial Funding Date: 5/94

Expiration: 4/96

FY 1995 Funding: $93,609

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

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.

The results of this study should indicate whether or not exposure of a developing rat to space flight will have an effect on its ability to have a normal immune response. This should provide information about the human immune system as well. In addition, the increased understanding of the development of immune responses could aid in the development of treatments for medical problems on Earth. For example, we may be able to find new ways to fight diseases in children on Earth.

In the past fiscal year, the flight study was carried out. Pregnant rats were flown on the Space Shuttle, and pregnant control rats were maintained in the animal enclosure module (AEM) on the ground. Additional control rats were maintained in standard vivarium housing. Experiments were carried out to determine the effects of flight on immunological parameters of dams, fetuses and pups. The ability of bone marrow cells of the dams to form colonies in response to granulocyte-macrophage colony stimulating factor was inhibited after space flight, but the colony forming cell response of fetus and pup liver cells was not inhibited after flight. Proliferation of spleen cells in response to mitogens was inhibited in flown adult animals compared to AEM controls but was not inhibited compared to AEM controls in cells obtained from fetuses and pups. Previous space flight studies indicated alterations in leukocyte subset distribution in adult rats. Preliminary analysis of the results of this study suggest that alterations in leukocyte subset distribution similarly occur in fetuses and pups. Cytokine

production profiles and immunoglobulin levels are currently under analysis. The results of this study indicate the same space flight-induced alterations in immune responses that occur in adults also occur in fetuses and pups, but others that are induced in adults are not induced in fetuses and pups.

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 of human childhood immunological disorders.

Publications, Presentations, and Other Accomplishments:

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Role of Thyroxine in Space-Developed Jellyfish

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-02

Initial Funding Date: 9/95

Expiration: 12/95

FY 1995 Funding: $23,850

Students Funded Under Research: 3

Flight Information:

Flight Assignment: ARF-2 (STS-82, 1997)

Responsible NASA Center: Kennedy Space Center

Task Description:

The metamorphosis process which enables the formation of ephyrae from polyps is influenced by a hormone, JF-T4 which is synthesized following iodine administration. Two groups of polyps in space (but not controls), formed ephyrae without iodine administration. In addition, in space, jellyfish ephyrae lost most statoliths and swam/pulsed abnormally. These findings suggest that the jellyfish hormone synthesis, utilization, or secretion may have been different in space as compared with ground controls. We therefore propose to measure the JF-T4 and T4 receptors in jellyfish polyps, strobilae and ephurae at different stages of development in space an on Earth. (c) The first year will be devoted to measuring the hormone and receptor in ground-based jellyfish and locating it in specific structures and cells of the different jellyfish cultures will be devoted to measuring the hormone and receptor in stages of the jellyfish at the light microscope and ultrastructural levels. Also, jellyfish cultures will be grown to provide animals for the flight experiment.

Funding for this study began September 19, 1995. In the interim between that date and December 31, 1995, we began building up our jellyfish cultures of polyps to provide numerous animals of high quality for the jellyfish experiment.

In 1995, we also began exploring modified fixation methods for the improvement of fixation of the jellyfish for electron microscope studies. These methods will be applied during testing jellyfish fixation in the flight hardware of the ARF (Aquatic Research Facility) to be used for the 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 organism on Earth as well as in

space. Thus far, little is known about the effects of thyroid hormone(s) on developing higher organisms in space partly because the time required for completion of mammalian development far exceed the current time periods 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: the differentiation of new structures such as graviceptors with statoliths and hair cells; the differentiation of a new neuromuscular (motor) system; and 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 structure in mammals, including human. Such an understanding could lead to prevention of hypothyroid-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-thuroxine, may be synthesized in greater amounts in space. If so, then thyroid hormone 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 counter-measures 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 space station or 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.

Effects of Microgravity on Tobacco Hornworm (Manduca Sexta) *During Metamorphosis*

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 89-13 OSSA IML-2

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: BRIC-04 (STS-70, 6/95)

Responsible NASA Center: Kennedy Space Center

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) and placed in a Shuttle mid-deck locker and recovered 9 days after launch. Examinations revealed suspended development in both flight or ground control organisms. Subsequent ground-based studies suggest that the arrested development in both flight and ground controls was a result of limited gas exchange into the canisters. To test this hypothesis, a series of experiments was run with altered gaskets and seals to the canisters. Collectively, the results suggest that an accumulation of CO_2 and/or depletion of O_2 arrested the development of the insects in the canisters. Follow-up testing in space flight with modified BRIC hardware is proposed to further study the development of the gravity sensitive development of tobacco hornworm.

On July 13, 1995, three BRIC canisters with 18 tobacco hornworm (*Manduca sexta*) pupae each were launched on board the Space Shuttle. Nine days after launch, the canisters were recovered and pupae were examined. Neither the flight nor the ground controls showed significant development. Half the pharate adults were bled immediately; the other half being returned live to the University of Arizona. Of the 27 remaining flight animals, 21 began developing after removal from the sealed canisters. These pharate adults were monitored and a few even completed development to the adult moth. Overall the flight and ground controls were in excellent agreement.

The preliminary hypothesis for the failure of both the flight and ground controls to develop was that a build-up of CO_2 in the canisters anesthetized the insects, causing them to become dormant. While preflight testing during the preceding year had not revealed any problems, the canister used for preflight testing was not precisely identical to those provided at the time of the flight experiment. The flight and ground-control canisters contained a gasket on both the top and bottom lids and were further sealed with vacuum grease. The canisters used for preflight testing contained a gasket on either lid and vacuum grease was not provided as a sealant. To test the effects of different degrees of atmospheric closure, a series of four experiments was conducted with the original BRIC canister used for preflight testing and with two of three ground-control canisters. In addition to testing for the effects of having gaskets in place with or without grease, we also tested for the possibility that new tubes in which the insects were housed may have had some deleterious effect.

As predicted the only conditions in which there was significant gas exchange in the canisters was there any adult development. Curiously, when grease or a gasket was placed inside the BRIC without grease or gaskets on the lids, the extent of the development appeared slightly delayed. But this was likely an anomaly reflecting a slightly slower developing group of insects at that time. There was no evidence that new insect support tubes had any effect. Therefore we can rule out the possibility that the tubes leached out any chemicals during the period when the BRICs were sealed. Nonetheless, it seems advisable to autoclave the tubes and to exclude any contact with grease or gaskets to avoid potential problems.

In the final analysis, it appears that the failure of the insects to develop was a consequence of either accumulation of CO_2 or depletion of O_2 from the well-sealed BRICs. Since sealing the BRIC would necessarily lead to a replacement of O_2 by CO_2 from respiration, it will be important to house the insects in BRICs from which the gaskets have been removed and to exclude vacuum grease from the protocol. Furthermore, it will be important to autoclave any new tubes generated for the reflight of the experiment. It is noteworthy, that wrapping the pupae in tissue inside of lexan tubes and cushioning the ends with moistened cotton seemed to have protected the insects from physical damage during the launch.

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.

Effect of Spaceflight on TGF-β Expression by hFOB Cells

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Funding:

Project Identification:

Solicitation: 93-OLMSA-04

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Expiration: 12/95

FY 1995 Funding: $93,293

Students Funded Under Research: 2

Flight Information:

Flight Assignment: 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 bed rest 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 for that protein was found to be expressed in bone at reduced level following space flight but that level was dramatically increased (within 24 hours) when normal activity was reestablished following space flight.

This experiment to be flown on STS-69 will determine that gene expression is reduced in cultured bone cells following space flight and 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. The cells 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.

Our efforts in FY95 were directed toward: 1) establishing the growth conditions for human hFOB cells which would allow us to achieve the major goal of the experiment which was to determine the effects of space flight and reloading on TGF-β expression on cultured osteoblast-like cells, 2) performing a realistic dry run at the Kennedy Space flight Center to test the flight hardware and practice the protocol under field conditions, 3) perform the space flight experiment, and 4) analyze the data. All of the goals leading to the space flight experiment were accomplished. Additionally, the flight experiment was a success. The results show that hFOB cells grow during space flight in a similar manner to on Earth. Interestingly, TGKF-βmRNA levels were increased 24hr following recovery, a similar increase following space flight was noted in skeletal tissues of rats following reinitiation of weight bearing. As a result of this project we are eager to perform a second space flight experiment to replicate our results and to extend them by investigating the response of bone cells at additional time points following recovery from space flight.

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 bed rest, 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.

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Kapelner, S.N., R.T. Turner, G. Sarkar and M.E. Bolander "(Poster presentation) Deletion mutation can be an unsuspected gel artifact." Miami 1994 Bio/Technology Winter Symposia: Advances in Gene Technology: Molecular Biology and Human Disease, Ft. Lauderdale, FL, February, 1994.

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Effect of Space Travel on Skeletal Myofibers

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No Co-I's Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-04

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 7

Joint Participation: NIH, WRAIR

Flight Information:

Flight Assignment: NIH-C5 (STS-72, 1995)

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 also will provide a rapid screening system for testing drugs to prevent muscle atrophy.

The recent development of a tissue culture incubator for middeck experiments [Space Tissue Loss (STL) Module] allowed the study of the effects of space travel on isolated skeletal myofibers. Avian skeletal muscle tissue cultures containing differentiated myofibers and connective tissue fibroblasts were flown for 11 days on STS66 (Nov., 1994). Metabolic rates, protein synthesis and degradation rates, and quantitative morphometry of the tissue were assayed. The rates of glucose utilization and protein degradation were accelerated during launch, indicating a launch-associated cellular "stress" response. Once in space, the metabolic and protein degradation rates returned to those of ground controls. Total protein synthesis rates at Day 8 of flight were identical to ground controls. Based on morphometric measurements, the skeletal myofibers in space atrophied 10% ($P<.002$, $N=250$) while interstitial fibroblast cell density increased 33% ($P<.001$, $N=60$) compared to ground controls. A "stress/injury" response associated with launch may initiate muscle tissue remodeling, activating connective tissue-forming fibroblasts at the expense of the myofibers. The results of this study support

the hypothesis that space travel can have a direct effect on skeletal muscle cells separate from any systemic effects on circulating growth factors. A second experiment is planned for Jan. 1996 (STS72) to confirm and extent these results.

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.

Publications, Presentations, and Other Accomplishments:

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Funding:

Project Identification:

Solicitation: AO-93-OLMSA-01

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-R3 (STS-72, 11/95)

Responsible NASA Center: Ames Research Center

Task Description:

No additional data was provided by the investigator for this research.

Water Purification in Microgravity by Freeze Separation

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: 199-61-17-02

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/97

FY 1995 Funding: $137,817

Students Funded Under Research: 0

Task Description:

Freeze Separation by water purification when applied to closed loop life support systems for space applications may replace current purification methods that have difficulty removing volatile organics and ammonia while using less power.

Boeing has developed a new technique for freeze separation that is compact and gravity independent to produce high quality water from waste streams. The freeze separation apparatus contacts the waste stream with a surface of uniform temperature and heat transfer characteristics creating a uniform layer of pure ice on the surface.

The research project is composed of two parts: ground testing and analytical modeling. Ground testing will utilize Boeing developed hardware to characterize the freeze separation process. Analytical modeling will use the test data to create a predictive tool for further process development.

The Water Purification in Microgravity by Freeze Separation Project objectives are 1) to determine energy and fluid transport parameters for simulation and prototype development; 2) to demonstrate the ability to create ice crystals with minimal occlusions, and 3) to quantify the thermodynamic separation efficiency of a gravity independent crystallizer.

The apparatus has been assembled and instrumented. The cooling jacket and chiller have been leak checked and the chiller set point temperature correlated to the actual cooling jacket surface temperature. The axial temperature profile of the cooling jacket were verified to be uniform during the check-out tests. The perforated tube was tested and modified until the flow distribution was deemed uniform and was subsequently integrated into the crystallizer. The crystallizer, chiller, pump, and tanks were integrated as a system and leak tested. The final system check-out was performed with deionized water verifying the freezing characteristics of the crystallizer.

Dr. Daniel Thomas has developed a detailed model of the system. The model considers mass and energy transport and has been used to establish crystallizer operating conditions.

Crystallization is a process commonly practiced by the chemical process industries. The operation may have utility in space applications to purify water from waste streams. Boeing has conducted internal research and development to demonstrate the feasibility of a low gravity water crystallization process, termed freeze separation. This technology could potentially provide a product water with lower light organic concentrations than single stage distillation while using less energy. Missions to Mars or a lunar base could take advantage of the cold of deep space to operate the process to drastically reduce the electrical energy requirements of water purification equipment.

Advanced Waste Management Technology Evaluation

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Funding:

Project Identification: 199-61-17-06 Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95 Expiration: 2/96

FY 1995 Funding: $167,092 Students Funded Under Research: 0

Task Description:

The overall objective of this project is the parametric evaluation of steam reformation as a candidate process for detoxifying and recycling biologic and other organic wastes . The intent of the steam reforming is to oxidize organic wastes to the inorganic carbon oxides, water and low molecular weight salts or nutrients. The inorganic carbon monoxide is easily converted to the dioxide with known technology (catalytic combustion or water shift) which can then be used by crop plants and/or processed through a Sabatier reactor.

Performance under this contract is on target. All apparatus has been designed, fabricated and debugged. Five test points of a 31 test point test matrix have been completed. The initial tests, conducted with fine grain cellulose (Avicel PH-200 from Rohm & Haas) as a model compound, have shown that approximately 70-80% of the cellulose mass is readily gasified at temperatures of 700-1400 (degree) F and that the residual char is readily gasified (completely in the case of cellulose) with the addition of only 5-6 mole% oxygen in 14000F steam. Without added oxygen, the digestion of carbonaceous chars is slow, where only half of the char was gasified by an additional 90 minute exposure to 1400°F steam; and the rate of the gasification reaction was observed to be decreasing with exposure time. Two quarterly progress reports have been submitted under this contract.

This work is targeted both towards near term and long term manned space exploration. For near term manned space endeavors, utilization of a detoxifying waste processing system could save much space and launch (and return) mass presently associated with waste storage; and for longer term manned space endeavors, a sophisticated waste processing system will be required to enable recycling biologic and other wastes back into plant and food values. (CELSS).

Advanced steam reformation, probably with 5 to 10% added oxygen to reach a point of near thermal neutrality also has potential for processing biological and/or chemical wastes back into innocuous inorganic compounds on earth or in remote stations (Antarctica) requiring rigorous preservation of a pristine environment. In either application, steam is a much better "moderating" component than the nitrogen in air (in an incinerative process) since it can participate in the oxidation reactions, generating hydrogen, and cannot participate in the production of toxic nitric oxides.

Publications, Presentations, and Other Accomplishments:

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Patent Approved, U.S. Patent #: 5,480,625, Birbara, P.J. "Enhancing Carbon Dioxide Sorption Rates Using Hygroscopic Additives."

Patent Approved, U.S. Patent #: 5,454,968, Birbara, P.J. "Flat Sheet Carbon Dioxide Sorbent."

Crop Production Optimization Using CO_2 Gas-exchange

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Funding:

Project Identification: 199-61-17-08

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $152,421

Students Funded Under Research: 12

Task Description:

We propose to use measurements of CO_2 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 will provide the basis for verifying and refining our energy cascade model of wheat productivity. We propose to extend our analytical techniques to soybeans and rice in the second and third years of the project. We also propose to examine the yield potential of a promising new wheat line.

We published the initial results of our analysis of physiological yield components (An approach to Crop Modeling with the Energy Cascade). This work led to the need for accurate measurements of canopy temperature. We have now modified commercial infrared transducers to achieve 0.1°C measurement accuracy of canopy temperature. These sensors should be useful to all groups doing NASA related plant research. We are now using canopy temperature as a primary input to transpiration models for regenerative systems. We have also completed the construction of a gas exchange system that uses state-of-the-art computer data acquisition equipment to simultaneously monitor and control 10 chambers. We are currently using this system to examine temperature and blue light interactions in soybean. Our models indicate that phasic temperature control is an important component in yield optimization and we plan to use this 10-chamber system to examine temperature interactions with other environmental factors. We are also in the process of formally releasing a new wheat cultivar that we developed specifically for controlled environments. To our knowledge, this is the first example of a genetic manipulation to improve our ability to explore space.

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.

Publications, Presentations, and Other Accomplishments:

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B. Bugbee, O. Monje, and B. Tanner. "Optimizing crop productivity: new sensors for measurement of CO_2, temperature, and air velocity, (Abstract)." Adv. Space Res., 1994.

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W. Doucette, and B. Bugbee. "Microcosm method for investigating the biodegradation of organic compounds in rhizosphere soils. (Abstract)" 9th Annual Hazardous Waste Remediation Conference, 1994. .

AI Software Development for Advanced Life Support

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Funding:

Project Identification: 199-61-23-17

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $96,329

Students Funded Under Research: 0

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.

Functional requirements were defined by a top-down approach that identified general and comprehensive advanced life support requirements and a bottom-up approach that identified specific CELSS Breadboard Facility (CBF) requirements. These requirements have been collected in a Functional Specification Document. Detailed requirements were defined by interviewing CBF system users and evaluating conceptual designs of a lunar base.

The functional requirements were evaluated to identify appropriate technologies for implementation, in particular assessing advanced automation technologies for cost-effective approaches. A system development plan was prepared based on high-payback tasks, where there is a near-term benefit for the CBF, consistency with CBF internal development plans, and available funding. Highest priority was and will continue to be given to tasks that are immediately useful to the CBF, have relevance to a general biological advanced life support system, and have potential for commercialization.

A software task, providing a tool for automating the CBF data validation process, was selected to initiate development and to provide insight into the implementation environment. This tool will initially provide operator assistance for the task, flagging anomalous data values that need human evaluation. As the validation process becomes better documented and understood, data will increasingly be dispositioned automatically. Development was started towards the end of the GFY.

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.

Publications, Presentations, and Other Accomplishments:

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Krauskopf, J., Drysdale, A.E., and Hendricks, D. "Controlled Ecological Life Support System Monitor and Control." Proceedings of the 31st Space Congress, Cocoa Beach, 1994.

Adsorbed Carbon Dioxide and Water Interactions and Maintenance of Low CO_2 Levels in Closed Environments

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: 199-61-12-12

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $125,400

Students Funded Under Research: 0

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 CO_2 found in the Earth's atmosphere (0.03%). Because of CO_2's physiological effects, the high level of CO_2 may make meaningful comparisons between ground and flight experiments difficult or impossible.

While a new specification has not yet been established, the CO_2 removal design in the current ISSA life support system is incapable of meeting a much lower CO_2 specification, given the CO_2 generation rate of four crew members. This is primarily due to the inefficiency of the adsorption technology used to remove the CO_2. 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 CO_2 on the adsorbents used in the current design. A regenerable, high-flow CO_2 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 CO_2 and water interact with each other when adsorbed on various hydrophilic and hydrophobic adsorbent surfaces and how the interactions affect the performance of CO_2 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 CO_2 removal from a humid natural gas stream, CO_2 concentration and control in closed environmental chambers, and air revitalization on long-duration passenger aircraft flights.

During the first year of the grant, work was initiated and proceeded along several lines as planned. These include background research, adsorption theory, and adsorption experiments. The following are the principal accomplishments in each of these areas:

Background Research
- Contract personnel were hired for the project.
- A literature survey of CO_2 removal techniques presently in use for all known applications (e.g., aerospace, diving, natural gas production, flue gas scrubbing, etc.) was completed; documentation is being prepared.

Adsorption Theory
- Several adsorption equilibria theories were reviewed for possible use in predicting CO_2/H_2O co-adsorption.
- A related joint research project with Southern Illinois University was initiated for development of a new, high efficiency/high accuracy computer algorithm for rigorously simulating multicomponent adsorption processors.

Adsorption Experiments
- On a related project, single- and multiple-component adsorption equipment was designed, fabricated, and used to measure CO_2 and H_2O co-adsorption on adsorbents used in the four-bed molecular sieve unit (Space Station CO_2 removal device)
- An automatic instrument was acquired for obtaining adsorption isotherms of gases and surface characterizations.
- Single component isotherms for CO_2, H_2O, and N_2 gases were obtained and physical property data were gathered for many of the materials to be used in the study.

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, modern passenger aircraft, vehicles on modern battlefields (such as tanks, helicopters, and personnel carriers), and underwater and high-altitude vehicles. If CO_2 removal is necessary for these applications, then it will probably also face the difficulty of efficiently removing CO_2 from humid air, the problem this research addresses. There are also industrial applications which require CO_2 removal from a humid gas stream, such as CO_2 scrubbing of natural gas. The benefits these applications could see from this research are mainly smaller and more energy-efficient ways of maintaining CO_2 at more desirable levels, which in turn would have positive impacts on human health and well-being and prices of industrial products.

Low pCO_2 Air-Polarized CO_2 Concentrator Development (Phase I of Space Station Experiment Development Study)

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No Co-I's Assigned to this Task

Funding:

Project Identification: 106-20-01-06

Solicitation: 93-OLMSA-07

Initial Funding Date: 11/95

Expiration: 6/97

FY 1995 Funding: $180,994

Students Funded Under Research: 0

Task Description:

The objective of this Space Station Experiment Development Study Program (Phase I) is to complete the effort required to verify the performance and applicability of the electrochemical Air-Polarized CO_2 Concentration (APC) process technology space missions requiring low CO_2 partial pressure (pCO_2), i.e., less than 1 mm Hg in the cabin atmosphere. This effort will be implemented by performing actual testing activities using multi-cell units (MCUs) for demonstration of the performance characteristics achieved in prior advanced electrochemical CO_2 removal technology study programs. The MCUs to be used are of an approximately one-person capacity (2.20 lb CO_2 removal per a 24-hr period) to demonstrate the technology at a readily scalable size, which will allow risk-free definition of a follow-on full-size Space Station Flight Experiment.

Since the contract was awarded in late 1995, the progress of the technical tasks has been limited to the following areas:

1. Start review of past work (internal and external).
    - Test data for various CO_2 removal systems published in the last 5 years.
    - Flight experiment data published in the International Conference on Environmental Systems (ICES) papers.
    - Technology evaluation data/report published by NASA organizations.
2. Identify requirements for critical hardware components of the Electrochemical CO_2 Separator (ECS).
3. Identify ECS electrode (anode) substrate material and its activation sources.
4. Prepare existing hardware, e.g., electrochemical cell modules and Test Support Accessories, for modification.

Potential areas where this technology will benefit the people of Earth include air revitalization for a permanent underwater exploration laboratory and air revitalization for extended (e.g., months) underwater military operations.

Enhanced Molecular Sieve CO_2 Removal Evaluation

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Funding:

Project Identification: 199-61-17-11

Solicitation: 93-OLMSA-07

Initial Funding Date: 1/96

Expiration: 1/98

FY 1995 Funding: $146,272

Students Funded Under Research: 0

Task Description:

The objective of this research is 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 type 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 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 current knowledge base for these adsorbents is limited for effective utilization of these materials on the design and development of CO_2 removal systems for future IVA and EVA missions. The proposed research will provide the required technical data that will enable improved CO_2 removal systems for regenerative life support systems to be developed.

During FY 1995 preliminary effort was made, including the following:
- analytical investigation of the test conditions required
- preliminary identification of the test plan required to achieve study goals
- identification of the test rig required for test
- investigation into resources and materials availability during the study performance

This effort will allow an efficient start of this new NRA in 1996. The kickoff for this program is proposed for January 11, 1996.

The goal of this research is to investigate a new technology for the selective removal of CO_2 from air at ambient conditions for space applications.

Selective CO_2 removal does have terrestrial applications, notably for food storage and some production plant cleanup; however, the most important gains may be: to facilitate man living in space, information about CO_2 selective adsorbents, bed design and operations to most enhance the performance of such materials, training and experience with alternative CO_2 removal technologies.

Additionally, removal of CO_2 from air is related to global warming and in the future, control of waste gas emissions of CO_2 may become necessary. This research will help define the chemical processes and conditions required for these types of control systems.

A Novel Method For Air Revitalization-CO_2 Removal From Air By a Pulsating Device

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Funding:

Project Identification: 199-61-17-07
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1995 Funding: $77,081
Students Funded Under Research: 5

Task Description:

A novel method for carbon dioxide removal from air is proposed. The idea is to remove large amounts of CO_2 from air and send it in the form of a concentrated gas to a bioconverting environment such as plant photosynthesis. In this proposal we will focus on the separation process and not on the bioconversion. The method that we propose 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 propose to test the theory with experiments and optimize the removal process. Further improvements are envisioned and based upon the mass transfer enhancement due to temperature gradients. This is the familiar Soret effect. Other innovative changes that are proposed include changing geometry and hydrodynamic conditions so as to effect a better separation between gases. This proposal concentrates on the separation mechanism and its optimization. The advantage to the space program is immediate as a new and novel means of air revitalization will then become available. Further, fundamental advances to the theory of gas dispersion by means of pulsating flows will be made.

Two major objectives have been accomplished. First, the theory for gas separation by pulsating processes was developed and the sensitivity with respect to geometry was investigated. It was found that geometries made up of multiple concentric annular tubes offered an advantage in separation of carbon dioxide from air at the expense of an increase in power. This has raised the question of optimization and that is what the current focus will be. Second, a sophisticated apparatus was constructed to verify the theory. The apparatus is flexible inasmuch as liquid separations and particle separations may also be studied in the future. The experimental work done this year can therefore affect future research if the scope of this project widens to include liquid and submicron particle separation.

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.

Publications, Presentations, and Other Accomplishments:

Beal, J. The effect of curvature on convective mass transfer via forced oscillatory flow, M.S. Thesis, Univ. of Florida. (1995).

Narayanan, R. "Mass transfer enhancement via forced oscillatory flow." American Inst. Chem. Eng., Annual Meeting, Miami, November, 1995.

Testing an Algae-Based Air-Regeneration System Designed For Use in a Weightless Environment

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Funding:

Project Identification: 199-61-17-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/97

FY 1995 Funding: $25,288

Students Funded Under Research: 4

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 will be set-up and tested on the ground with a variety of unicellular algae. A number of basic questions concerning the development of the tube will be addressed. Of particular interest at this stage will be the rate of photosynthetic carbon uptake per tube.

This period saw the completion of some of the initial steps in the testing process. In summary, ceramic tubes (nominal pore size 0.3 or 0.4 μm) were inoculated by dripping infusions of algae (*Chlorococcum*) on their outer surfaces. The tubes were then incubated in a plexiglass glass chamber within an environmental growth chamber. Liquid medium was pumped though the tube for 5 minutes each day; the positive pressure caused by the type of pump resulted in a mass flow of filtered medium through the walls of tube and precluded continuous pumping. Under these conditions, visible growth appeared on the surface of the tube in 2 to 3 weeks and could be maintained for at least an additional 3 weeks; contamination did not appear to pose a serious threat. It should also be noted that one tube was allowed to dry completely while the lab was moved to Valdosta State University (August to September), but the organisms remained viable.

As the result of these preliminary steps, the following improvements in the system have been made. 1) The tubes are now inoculated by painting the exterior surface with a cotton swab dipped in an infusion of algae and medium. 2) For routine screening of cultures, medium is circulated through the tubes using a one-way gravity-flow system. For long-term growth experiments, medium is circulated at low flow rates by means of peristaltic pumps attached at the down-stream end of the tubes; this reduces the pressure of the liquid in the tubes. 3) The tubes are incubated in polypropylene chambers; these reduce the amount of light reaching the algae, but are easier to work with than glass and plexiglass.

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.

Space Experiment on Tuber Development & Starch Accumulation for CELSS

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-61-17-24

Solicitation:

Initial Funding Date: 2/95

Expiration: 2/97

FY 1995 Funding: $75,410

Students Funded Under Research: 0

Task Description:

Tuber production of white potatoes is of major importance for providing energy-rich carbohydrates in controlled ecological life support system (CELSS). Starch represent 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, is questioned particularly in microgravity but possibly even under reduced gravity on Moon and Mars. This experiment is proposed to study the effect of weightlessness on accumulation of energy-rich starch in potato tubers using excised leaves on which the axillary buds can be induced to develop small tubers in 10-14 days. This provides an easily controlled system for study of tuber growth and starch production in microgravity on the space shuttle. It is planned to fly this experiment in conjunction with the flight of ASTROCULTURE ™ plant growing unit being developed by the Wisconsin Center for Space Automation and Robotics and is scheduled as a middeck locker experiment on the 16 day flight of USML-2 mission in September 1995. The study will determine the rate of growth of tubers under microgravity, concentrations of starch and other carbohydrates, enzyme activities associated with starch synthesis and starch degradation, and the characteristics of the organelles that accumulate starch in the tubers. The determinations will be made on tubers from both flight and ground control experiments. Investigations prior to flight will be concentrated on obtaining uniform tuberization on cuttings, ensuring that plant growth is not restricted within the ASTROCULTURE ™ flight unit, and obtaining the baseline growth, biochemical, and anatomical data for the plants to be flown in the USML-2 mission. The results from this project will provide important information on the whether limitations to tuber development and starch accumulation in potatoes are manifested under reduced gravity of space.

The second year of the this project has been directed toward completing preflight research. The preflight research from March to October 1995 involved investigating cutting response in prototype packages under simulated flight protocols. Two small chambers were constructed with inside dimensions similar to the flight unit and mounted onto root manifolds that contained porous tubes for controlled watering and that could be filled with particulate media to support the base of the cuttings. The chambers were radiated with LED light banks containing red and blue LED lamps in the same proportions as in the flight unit. These chambers were maintained in a reach-in growth chamber and each fitted with a fan to pull air from the chamber through the growth area and exhaust the air back into

the chamber. The growth chamber was maintained at the temperature and humidity levels programmed for the flight unit. During these preflight studies procedures for handling cuttings, planting in the media, and effective separation of the five leaves in the package were developed. Also, it was determined that cuttings survived best by using arcillite rather than xeoponics as a media, and that watering with distilled water was preferable to using nutrient solution.

Procedures were also developed for holding cuttings for 12 hours in hand-held coolers at 5° C to duplicate the procedures to be used for ground control cuttings that would be harvested at KSC and transported to Madison for the postflight control study. (This same procedure was followed for cuttings used in the USML-2 flight to insure similarity between flight and control cuttings.) Cuttings at different stages of development were analyzed to obtain baseline data on the development of the tubers and the levels of carbon dioxide and enzymes in the different tissues of each cutting. Particular study was made of the rate of tuber differentiation over the first 72 hours after removal of the cutting from the plant to establish how long a delay could be tolerated between harvesting of the cuttings and launch of the shuttle.

On June 15, plantings were initiated at Hangar L at Kennedy Space Center and repeat plantings were made at two week intervals after that date. The first group of plantings at KSC was undertaken to insure that plant growth at KSC closely duplicated growth in the Biotron at the University of Wisconsin. Plantings made after the first week of July were maintained for flight and postflight ground control experiments. Plantings at two week intervals were also undertaken in the Biotron at Madison to provide backup plants if necessary for the KSC plants. The flight was scheduled for September 14, but mechanical problems and weather scrubs delayed the launch until October 20.

Tuber production is of major importance for providing energy-rich carbohydrates for controlled ecological life support systems (CELSS). Starch represents the major source of energy in foodstuffs derived from plants, 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, may be seriously limited in microgravity and possibly in reduced gravity on the Moon and Mars.

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.

Biochemical Capture and Removal of Carbon Dioxide

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Funding:

Project Identification: 199-61-17-12 Solicitation: 93-OLMSA-07

Initial Funding Date: 8/95 Expiration: 8/98

FY 1995 Funding: $195,950 Students Funded Under Research: 0

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 CO_2 from respiratory gases. Our second objective is to develop a method for the on-orbit regeneration of the enzyme-matrix CO_2 extraction system to support long-term space missions.

The program was operative only for the last two months of FY 95. During this time we made a further evaluation of the two alternatives presented in the grant application. We updated our literature review and became aware of new work in molecular biology relevant to production of the key enzyme. We identified additional recent references which reported cloning several isozymes of the enzyme. These data supported our resolve to use cloned and genetically modified enzyme as opposed to using new immobilization techniques with native purified enzyme. To this end we explored the availability of enzyme clones from the identified sources. In FY 96 we gained access to key clones. The basic question of whether CA could be expressed in *E. coli* has been answered in the affirmative.

We also explored new developments in immobilization methods and in conference with our consultants elected to continue with nylon surface modified to chelate metals as our immobilization substrate. In FY 96 we undertook additional steps to expand our capabilities in this area.

The application listed five specific aims. The first was to recombinantly engineer the enzyme and develop a DNA expression system for it using *E. coli*. The second was to immobilize the enzyme to a carrier. During FY 95 we set up the conditions to realize these two specific aims. In FY 95 we solidified our approach and capabilities in both of these areas. In FY 96 we expect to complete realization of these objectives.

The principal possible medical application is development of closed cycle anesthesia machines for gaseous anesthetics. This technology is also applicable to closed environments in addition to spacecraft. Examples include carbon dioxide cleansing systems applicable to closed environmental life support systems including hazardous materials handling, mine safety, aircraft and submarines.

A longer term Earth impact of this project is expected to be in scale up of the system as a means of capturing carbon dioxide currently released into the atmosphere. This should be important for reduction in greenhouse gases. Point sources account for more than one third of all of the carbon dioxide produced. We anticipate capturing a meaningful portion of this gas. Economic availability of CO_2 will result in development of additional new technologies for applying the available CO_2 to other uses, such as plant growth stimulation.

CELSS Crop Simulations for Systems Engineering and Productivity Optimization

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: 199-61-17-23

Solicitation:

Initial Funding Date: 9/95

Expiration: 8/00

FY 1995 Funding: $119,504

Students Funded Under Research: 0

Task Description:

The proposed research will continue 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. The proposed research takes as a central objective 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 will be to continue the strong collaboration established over the 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, continue 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 previous 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.

Work has been completed on modifying the Ceres- Wheat model based on the experimental data from Utah State University. In an important milestone, we have demonstrated that the field models can indeed be adapted to the controlled environments, given several important modifications. These modifications have so far included: a photosynthesis routine sensitive to CO_2 levels, temperature, and

diffuse light; a decreased partitioning to roots, a non-limited leaf expansion in early stages; an increased specific leaf area, and other changes. All these are reasonable and highly justified, given knowledge about plant physiology. As a result, we now have a wheat model that is successful at simulating the life cycle development of the NASA wheat. The model provides outputs for final biomass, harvest index, leaf area index, grains per square meter, average quantum yield, and other significant, experimentally-measured parameters. We have begun work on using the modified Ceres- Wheat model to explore the potentials for phasic control, focusing on temperature changes at stage boundaries in the life cycle. We have also begun applying the Soygro model to the soybean data from Kennedy Space Center, having learned from the work with wheat what to expect and thus how to approach the sequence of modifications.

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 work 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 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 possible on a near-future Earth.

Publications, Presentations, and Other Accomplishments:

Meleshko, G.I., Shepelev, Ye.Ya., Amerner, M.M., and Volk, T. "Biological and life support systems. In: Life Support and Habitability, vol. 2 of Space Biology and Medicine." Edited by: Sulzman, F.M., and Genin, A.M. AIAA/Washington, D.C., pp 357-394, 1994.

Tubiello, F. "Simulation of the effects of CO_2, climate change, and controlled environments on wheat growth and development." Ph.D., New York University, June 1995.

Tubiello, F.N., Volk, T., and Bugbee, B. "Simulating radiation-use efficiency for wheat grown in controlled environments under ambient CO_2 (Abstract)." American Society of Agronomy Annual Meeting, St. Louis, MO, November 5-10, 1995 .

Tubiello, F., Rosenzweig, C., and Volk, T. "A modified Ceres wheat model to simulate the interactions of CO_2, temperature, and management practices." Agricultural Systems, vol. 49, 135-152, 1995.

Volk, T. "An energy cascade model for analysis and prediction in gas exchange experiments of wheat growth (Abstract)." Second International Conference on Life Support and Biosphere Science, Huntsville, AL, 21-23 February, 1994.

Volk, T. "New developments in modeling wheat in controlled environments." Presentation at NASA Ames Research Center, 10 August, 1995.

Volk, T. "Advances in modeling wheat and soy." Presentation at Kennedy Space Center, November 14, 1995.

Volk, T., Bugbee, B., and Wheeler, R.M. "An approach to crop modeling with the energy cascade." Life Support and Biosphere Science, vol. 1, 119-127, 1995.

Acoustic Bone Mass and Trabecular Property Measurements

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No Co-I's Assigned to this Task

Funding:

Project Identification: 106-20-08

Solicitation: 93-OLMSA-07

Initial Funding Date: 4/95

Expiration: 3/98

FY 1995 Funding: $107,230

Students Funded Under Research: 1

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.

Two experimental setups were constructed and calibrated. One is for subsonic and another one is for ultrasonic measurements. The series of tests were performed with both ultrasonic and subsonic techniques. Ultrasonic measurements were accomplished using phantoms and bovine bones. The nonlinear effect of modulation of the high frequency (200 - 300 kHz) probe wave with the low frequency (20 kHz) pump wave has been investigated. The work is under way to study quantitative correlation between modulation effect and trabecular bone density. While ultrasonic measurements can deliver information about the microstructure of bone, subsonic technique is intended to provide information regarding the overall mass of bone. Subsonic measurements with a simulated bone and human tibia (*in vivo*) proved the concept of the proposed method. The frequency range was determined, in which the effect of damping does not interfere with the measurements. The qualitative correlation between the measurements and bone mass was observed. The future study will be directed toward investigation of quantitative correlation between measurements and bone parameters and determination of precision of the method.

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.

Monitoring Physiological Variables with Membrane Probes

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Co-Investigators:

No Co-I's Assigned to this Task

Funding:

Project Identification: 106-20-01-03

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 8/96

FY 1995 Funding: $99,905

Students Funded Under Research: 0

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 will be developed to study some specific electrolytes (sodium, potassium, and chloride), hormones (aldosterone corticotropin [ACTH] and antidiuretic hormone [ADH]), 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 will be determined to validate the use of subcutaneous samples in place of blood samples in physiological studies. The rat hind limb suspension model will be 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 will also be developed to study the effects of hind limb elevation on changes in glucose and lactate within the muscle. On-line monitoring systems will be developed for the metabolites. Enzyme electrodes will be adapted for monitoring of glucose and lactate and will be incorporated into a low dead volume flow cell. Ion selective electrodes for electrolytes will be tested for possible use in on-line sensors.

The first objective of this project is to develop suitable microdialysis (MD) and ultrafiltrate (UF) probes for measuring the analytes of interest in this project. The analytes are sodium, potassium, chloride, vasopressin, ACTH, aldosterone, glucose, and lactate. The tissue sites to be studied are subcutaneous and muscle, using the head-down suspended rat model of microgravity.

Subcutaneous MD and UF probes have been constructed and are being tested *in vitro* for the analytes of interest. The UF probe consists of three 12 cm ultrafiltration fibers in a looped configuration attached to microbore tubing. The subcutaneous microdialysis probe consists of a single 5 cm fiber connected at each end to microbore tubing. The same hollow fiber material was used for each probe. For the microdialysis probes a flow rate of 2 L/min was used. The *in vitro* recoveries for the analytes which have been completed are shown below. *In vitro* recovery studies on the remaining analytes are in progress.

Table I: *In vitro* recoveries.

Analyte	UF-3-12		DL-5	
Sodium	101%	2%	101%	2%
Potassium	94%	13%	106%	4%
Chloride	96%	4%	95%	7%
Lactate	94%	5%	95%	7%

These recoveries verify that the UF and MD probes will be suitable for sampling these analytes in *in vivo* studies. From previous studies it is known that the UF probes will also be suitable for glucose, and preliminary data indicates that the MD probe will also be suitable for glucose.

The second objective of this study is to develop a head down suspension system for rodents. This system will allow for continuous sampling from implanted probes, a jugular catheter (for a 3 day control period), a two week suspension period, and a one week recovery period. The configuration which has proved most successful to date consists of a full-body jacket. A collar is placed on the animal and the jacket is attached to the collar to prevent escape forward. Two straps go around the hind legs to prevent escape from the back of the jacket. Further modifications will be tested to increase security and comfort for the animal. For the rest of the system the BAS awake animal system was used and modified by adding heights and weights to achieve suspension. At this time, several rats with subcutaneous MD and/or UF probes have been suspended with continuous sampling. The data from these studies has been partially analyzed. There is insufficient data at this time for statistically valid conclusions, however, some general observations can be given. Average concentrations and standard deviation of ultrafiltrate analytes under different conditions are listed in Table II.

Table II. Subcutaneous ultrafiltrate concentrations.

Analyte	Anesthetized		Baseline		Suspended		Recovery	
Sodium	135	4 meq/L	136	6	141	5	134	
Potassium	3.9	1.6 meq/L	3.8	0.6	4.3	0.2		3.5
Chloride	115	6 meq/L	110	5	104	5	111	4
Lactate	18	7 mg/dL	36	10	59	14	28	
Glucose	199	37 mg/dL	117	45	166			

The probes are implanted under Ketamine-Xylazine anesthesia which affects some analyte concentrations. Sodium and potassium concentrations are not affected by the anesthesia. Chloride is slightly elevated and lactate is slightly decreased. Glucose is significantly elevated by the anesthesia.

Suspension in a head down position results in an increase in sodium and potassium and a decrease in chloride. These ions return to baseline during the recovery period. Lactate and glucose are also elevated by head down suspension. Preliminary data indicated that glucose and lactate return to baseline or below during the recovery period.

Measurement of electrolytes was done with commercially available ion sensitive electrodes (ISE). For chloride a spectrophotometric method has also been used as a cross reference. Discrete samples were collected and analyzed with the electrodes with the goal of incorporating these electrodes into an on-line real time sensing arrangement. It appears at this time that the commercial potassium and chloride electrodes do not function well enough to be incorporated into an on-line system. Improved chloride and potassium ISEs will need to be developed for this function. More work needs to be done to determine if the sodium electrode can be used for an on-line system.

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 inlet and outlet tubing of the amperometric flow cell were compatible with the microdialysis tubing, and the testing conditions for the detector, such as perfusion solution and flow rate, were typical of microdialysis experiments.

The GOX electrode was prepared by coating the electrode surface with the enzyme and polymer mixture solution followed by a cellulose acetate and Nafion over-coating. The over-coating on the enzyme electrode served as mass transport limiting membranes for better linearity. During *in vitro* tests of the glucose sensor, the average 0-90% rise time of the response was 2 min. The electrode gave a linear response to 0.1-20 mM glucose concentrations, which covers the clinically relevant glucose concentrations. The electrode exhibited poor sensitivity to glucose concentrations below 0.1 mM due to oxygen competition with the redox polymer for GOX. After 72 hours of continuous use, the electrode lost 50% of its sensitivity. However, the electrode was still usable and the linearity of the response did not change. The system was tested with ascorbic acid, uric acid, and acetaminophen which can interfere with some oxidase based electrochemical sensors. There was no interference form uric acid, and there were minimal interferences from both ascorbic acid and acetaminophen which should be tolerable.

The LOX electrodes had a response time of 3 minutes, and lost 50% of their sensitivities after 24 hours of continuous operation. The LOX electrode showed no responses to 0.5 mM uric acid and 0.1 mM ascorbic acid solutions. However, it gave 233 nA/mM oxidation current for acetaminophen solution. Further efforts are underway in our lab to eliminate the acetaminophen interference in the LOX modified amperometric detector.

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 limit to 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 in different tissues. Therefore, membrane probes offer a method for studying metabolism in different tissues and obtaining better understanding of physiological and pathological processes.

Assessment of the Effects of Chronic Microgravity on Ventricular Mass by Three-Dimensional Echocardiography

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No Co-I's Assigned to this Task

Funding:

Project Identification: 106-20-08

Solicitation: 93-OLMSA-07

Initial Funding Date: 3/95

Expiration: 2/97

FY 1995 Funding: $92,805

Students Funded Under Research: 0

Task Description:

Our objectives are: 1) To assess the effects of chronic microgravity on cardiac mass (myocardial volume) by three-dimensional echocardiography. 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 can not 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: Left ventricular adaptive changes to chronic microgravity results in decreases of myocardial mass proportional to decreases in circulating blood volume, plus 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 locaters is being undertaken, prior to other planned developments. Comparison of the effectiveness and appropriateness of acoustic, electromagnetic and mechanical locaters for this purpose is being performed. Equipment is being acquired and prepared for testing in an integrated system. Computer software for control of this apparatus is being written. When this is complete, testing will begin.

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.

Fully Implantable Integrated Silicon Biotelemetry

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Funding:

Project Identification: 106-20-08 Solicitation: 93-OLMSA-07

Initial Funding Date: 3/95 Expiration: 2/98

FY 1995 Funding: $194,232 Students Funded Under Research: 2

Task Description:

We are developing 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 generated by neurons, blood pressure, core body temperature, and multi-axis acceleration 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.

In the first year of this project we have made important progress in several areas. We committed much of our time and resources during the first eight months of the project to the design, fabrication, and testing of a micromachined silicon-based capacitive pressure sensor which is to be used by investigators at NASA Ames for monitoring pressure fluctuation during delivery. Although this task was not part of the original project proposed to NASA, the need for such pressure sensors by NASA Ames and our expertise in the development of micromachined pressure sensors convinced us and NASA that we should exert some effort at developing these pressure sensors. We have now fabricated and tested the first prototype of these sensors and are ready to deliver these prototypes to our counterparts at NASA Ames for their testing. In spite of the time and effort that this task took, we were also able to make substantial progress towards the development of the original fully implantable systems as stated in our original proposal. We have now identified and developed our approach to the design of the overall electronic system, the package that can hold the electronics and interface with the sensors, the types of sensors and their fabrication technology, and the bidirectional wireless communication and telemetry link. In each of these areas, the potential challenges and necessary technologies have been identified. In the coming year we will design, fabricate, and test the first prototypes of the circuits and systems needed in each of these areas, as well the sensors, and will assemble the first complete system for testing in animal models.

We have now developed an architecture for the system electronics, functions, performance requirements, and telemetry requirements of the implantable module. We have determined that we will have to design and fabricate both the digital and analog portions of this system, instead of using commercial microcontrollers, and we have started efforts in the selection of the most appropriate technologies and circuit techniques for doing so. The most important requirements in this multi-channel system are low

power and high speed. The system will be capable of measuring data generated by fifteen sensor channels (8 neural at a bandwidth of 5kHz per channel, two pressure, one temperature, three acceleration, and one EKG/EEG channels). The design of the analog amplifiers and the on-chip analog-digital converter is the most challenging. CMOS switch-capacitor AD converters and dynamically-switched CMOS amplifiers are being considered for the first system. In the coming year a complete system will be designed, simulated, and fabricated for use in the implantable module.

We have also tailored our approach to the implementation of the other components of the implantable module. Various sensor data will be transmitted to an external receiver using a frequency-multiplexed ASK telemetry link operating at around 200MHz. All the elements of the simple Colpits oscillator, which will form the transmitter, except for the active transistor will be integrated on a single chip to be fabricated in our facilities. Program data and power-on commands will be transmitted to the implant using an array of low power electrostatic/electromagnetic switches that are formed through micromachining technologies.

A modular package is being developed to house the system electronics and the individual sensors. The goal is to develop a package for the overall system that allows interfacing to sensor modules that are removable and replaceable. We have devised a technology and developed an approach that will hopefully accommodate this requirement. This packaging approach will be implemented and tested in the coming year.

In summary, during the first year of this project we have answered many of the questions and issues regarding system electronics, data telemetry and bidirectional communication, packaging, and selection of sensor types, and have identified technologies with which we can best satisfy the requirements of low power, small size, high bandwidth, and long-term stability. In the coming year we will design and fabricate these various components and will implement and test the first prototype multichannel telemetry system.

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 developed 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 are 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.

Pulse Tube Refrigeration New Techniques for Improving Efficiency

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Funding:

Project Identification: 106-20-08 Solicitation: 93-OLMSA-07

Initial Funding Date: 4/95 Expiration: 3/98

FY 1995 Funding: $100,000 Students Funded Under Research: 0

Task Description:

The work proposed here consists of three phases, each of one year in length. Phase I consists of evaluating tapered regenerator geometries as a means of improving the efficiency of the pulse tube refrigerator. First the effects of tapering will be evaluated using NIST REGEN3.1 software. Second appropriate regenerators will be constructed and incorporated into a pulse tube refrigerator for evaluating the refrigerator performance and efficiency. For phase II we propose to develop methods to employ multi-staged pulse tube refrigerators. The appropriate candidate methods will be selected, constructed and experimentally evaluated. Additionally any new ideas or concepts resulting from phase I will be incorporated. In phase III a method to continuously stage a pulse tube refrigerator will be employed, constructed and experimentally evaluated.

The pulse tube refrigerator offers many advantages over the Stirling refrigerator because there are no moving parts at the cold end as there is in the Stirling refrigerator. In the last few years the efficiency of pulse tube refrigerators has nearly equalled that of Stirling refrigerators. Improvements in efficiency in both types of refrigerators are continually being made. In order for pulse tube refrigerators to compete with Stirling refrigerators for some applications where efficiency is of paramount importance, further improvements in efficiency are needed in the pulse tube refrigerator. This need is particularly important at higher cold end temperatures where the intrinsic efficiency of the orifice pulse tube refrigerator decreases.

Since it is the cold head that is different between Stirling and pulse tube refrigerators, we have focused our attention on the losses in this part of the refrigerator, that is we have ignored compressor losses since they would be the same for both refrigerators. The dominant cold end losses in the pulse tube refrigerator are the inefficiency of the pulse tube in transporting enthalpy and the ineffectiveness of the regenerator in blocking all enthalpy flow through it. We also consider axial thermal conduction in the regenerator as part of the regenerator loss. For an 80 K single stage pulse tube refrigerator the pulse tube loss is typically about 40% of the maximum available refrigeration power at the cold end (PV work flow). The regenerator ineffectiveness and conduction contribute a loss of about 30%. Real gas

effects (of which we have little control) contribute a loss of about 5%. Thus the net refrigeration is only about 25% of the maximum available.

Progress during FY95 of this contract includes studies of both the pulse tube and regenerator losses as a function of temperature. Measurements of the pulse tube efficiency were made using a pulse tube refrigerator that had a compressor PV power of about 50 W and operated at 50 Hz. At 80 K the pulse tube loss was about 46%, but this decreased to about 18% at 250 K. The NIST computer code REGEN3.1, which is used to model regenerators, was used to study the effect of temperature on the regenerator loss. For a 45 Hz system at 2.0 MPa average pressure the total regenerator loss at 80 K was 25% of the cold end work flow. At 250 K this loss decreased to 3.7% for the same regenerator geometry. The lost power due to pressure drop in the regenerator varied from 27% of the compressor PV power at 80 K to 26% at 250 K. At 250 K the only significant detrimental effect of the regenerator is the lost PV power. In order to improve the overall system efficiency at 250 K the pressure drop should be reduced significantly. Unfortunately, the system efficiency at 80 K will decrease since the design was optimized for that temperature. It is clear that to cover the entire range from about 75 K to 250 K, a compromise in the optimum design must be performed. Reducing the pressure drop without a serious increase in the low temperature regenerator loss at 80 K is currently being studied in this program. The use of tapered or stepped regenerators is being investigated for use over a wide temperature range.

The NIST computer model REGEN3.1 has been modified in FY95 to handle a tapered regenerator. In addition to a position dependent cross-sectional area the program can now handle variations in porosity, hydraulic diameter, and thermal properties. In practice the tapered geometry may be approximated by a stepped regenerator. In FY96 the model will be used to optimize the design of a stepped regenerator. A pulse tube apparatus was modified during FY95 so that it could be used to compare the performance of straight and stepped regenerator. Results from those tests will be compared with our model predictions.

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, 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 Dept. 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.

Multigas Sensor for Advanced Life Support

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Funding:

Project Identification: 106-20-08
Solicitation: 93-OLMSA-07
Initial Funding Date: 11/95
Expiration: 11/97
FY 1995 Funding: $95,239
Students Funded Under Research: 1

Task Description:

The project was started on the 3rd of November 1995. During the months of November and December 1995, and January 1996, considerable progress was achieved on the project tasks. Experimental techniques were developed to conduct meaningful studies on the project objectives. A three compartment laboratory cell with nonaqueous electrolyte solution with high electrolytic conductivity and wide electrochemical stability window was used in the cell. The electrode materials chosen for the detection of oxygen (O_2),humidity (H_2O) and carbon dioxide (CO_2) are: gold sensing electrode, platinum counter electrode and silver quasi-reference or platinum pseudo-reference electrode. The cell with the electrolyte and the electrodes was assembled in a glove bag with argon gas flowing. The instrumentation used was PAR Model 173 potentiostat/galvanostat with PAR Model 175 Universal Programmer and Keithly 175 autoranging multimeter interfaced with a McIntosh computer to run electrochemical experiments, data collection, and data reduction. After de-aerating the electrolyte solution with argon gas, linear scanning voltammetry was run to establish the base line from 0 to - 2.5 V vs Ag or Pt reference. Then linear scanning voltage experiments were run with a known concentration of oxygen gas in the solution of the central compartment. A well defined current peak was obtained at about -1V vs pt or -.8V vs Ag reference. Similar experiments were run with a known concentration of carbon dioxide gas in the central compartment. A well defined current peak was obtained at about - 2.2V vs pt or 2.3V vs Ag reference.

Experiments were conducted using both oxygen and carbon dioxide in the solution at the central compartment. Current-voltage peaks were were obtained almost simultaneously, one current peak appearing at about -.85V vs Ag corresponding to oxygen and another current peak appearing at about -2.3V vs Ag corresponding to carbon dioxide.

Experiments were conducted using oxygen and very small amount of water in the solution at the central compartment. Current -voltage peaks were obtained almost simultaneously, one current peak appearing at about -.85V vs Ag corresponding to oxygen and another broad peak appearing around 1.8V vs Ag. corresponding to water reduction. These experimental results clearly show that the electrochemical sensor based on nonaqueous electrolyte solution is capable of detecting oxygen, carbon dioxide and water. This sensor can also function as a multigas/vapor sensor.

This unique electrochemical sensor technology based on nonaqueous electrolytes has the potential for long life, low cost and reliable performance with multigas/vapor detection and monitoring.

This sensor has applications for monitoring toxic gases and vapors at homes and at work place. The sensor has applications in environmental monitoring, in industrial process monitoring and control and in medical diagnostics.

Publications, Presentations, and Other Accomplishments:

Smith, Jones, and Reed, Working in Space. J. Am. Phys., vol. 23, no. 5, 2-56 (1995).

New Statistical Methods for Immunoassay Data Analyses

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Funding:

Project Identification: 199-70-17-19
Solicitation: not applicable
Initial Funding Date: 6/95
Expiration: 5/97
FY 1995 Funding: $137,889
Students Funded Under Research: 2

Task Description:

The broad, long-term objectives of this project are: (1) to simplify biospecimen immunoassay procedures on manned space missions and at ground testing sites by using more efficient and more accurate statistical methods for immunoassay data analysis and assay quality control; (2) to develop more accurate statistical methods for immunoassay data analysis by combining new advances in Bayesian statistical theory with more accurate mathematical models of the physical and chemical properties of the immunoassay system; (3) to reduce in-flight biospecimen sampling and storage requirements, and to help ensure the validity and interpretability of experimental data.

The specific aims are to show that: (1) the dose-response curve based on the mass-action law is an accurate and reliable model for use in routine immunoassay data analysis; (2) the relative error model (REM) provides an accurate, interpretable description of assay experimental error; (3) combined, the mass-action dose-response curve (MADRC) and the REM give much more accurate model of immunoassay behavior than the widely used 4 parameter logistic model; (4) Bayesian statistical theory provides a comprehensive framework for immunoassay data analysis which avoids the large-sample theory approximations and justifications of current methods and into which the MADRC and REM can be easily incorporated. (5) a numerically efficient, immunoassay data analysis software package based on these models and using the Bayesian framework can be implemented on a personal computer for easy transport on space missions 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 analysis in any clinical or research laboratory.

The specific accomplishments of the research to date include: 1) formulation of a new, conceptually unified yet practical approach to immunoassay quality control, calibration and measurement based on a Bayesian statistical paradigm. 2) implementation and testing of a Monte Carlo Markov chain algorithm to carry out the new Bayesian statistical paradigm. 3) formulation and testing of a new,

conceptually sound and computationally feasible definition of the immunoassay minimal detectable concentration This new definition was used to study the Abbott microparticle capture enzyme immunoassay for prostate-specific antigen (PSA). It was shown that this assay has a minimal detectable concentration 4 to 7 times larger than stated and that as a consequence, the ability of the assay to measure reliably small concentrations of PSA in order to detect early recurrences of prostate cancer is probably overstated. 4) development of a software package written in C to perform immunoassay data analysis based on a new Bayesian statistical paradigm. The methods are appropriate for any ligand-based immunoassay system.

The research represents the development of new statistical techniques to analyze immunoassay data. These methods should have broad application in clinical and laboratory medicine because immunoassays are the most widely used procedure for measuring the concentrations of analytes in biological specimens. The primary benefits of these new methods will be had 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.

Publications, Presentations, and Other Accomplishments:

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Orthostatic Intolerance - Short Flights

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Funding:

Project Identification: 199-70-11-20

Solicitation: 93-OLMSA-07

Initial Funding Date: 1/95

Expiration: 1/97

FY 1995 Funding: $77,688

Students Funded Under Research: 0

Responsible NASA Center: Johnson Space Center

Task Description:

Reduced orthostatic tolerance has been observed in crew members after space flights. Symptoms have ranged from increased heart rate to presyncopal episodes. In the U.S. space shuttle program, most crew members have been tested for orthostatic intolerance using the stand test before and after space flights. The various parameters such as heart rate, blood pressure and echocardiographic information collected during these tests are available, and analysis have been carried out periodically to answer specific operational questions. However, no analysis has been carried out to examine individual risk factors contributing to orthostatic intolerance in crew members from this data. There is a need to integrate all the existing information into a well-defined database, and examine the epidemiological aspects of orthostatic intolerance. This project is designed with the main objective to evaluate individual risk factors for orthostatic intolerance after space flights. In order to examine the cumulative incidence of orthostatic intolerance, we propose to use the product-limit method of Kaplan and Meier survival curves and utilize the information on the time to orthostatic intolerance. To examine the individual risk factors, we propose to use a case-control approach, where unconditional logistic regression analysis will be used to assess the influence of multiple variables on the risk of orthostatic intolerance. Such an analysis will offer valuable insight into the characteristics of orthostatic intolerance after space flights. The results from these analyses could also be used for operational decision-making and treatment strategies.

No additional data was provided by the investigator for this research.

NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis

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Funding:

Project Identification: 199-18-17-13

Solicitation:

Initial Funding Date: 1/94

Expiration: 1/97

FY 1995 Funding: $388,257

Students Funded Under Research: 12

Task Description:

The Center will contain two components, a research component and a training component. The goal of the center's research program is to develop an integrated quantitative approach to understanding the effects of space flight on cardiovascular function. To accomplish this objective, we will: a) Develop computer models of cardiovascular function including aspects of pulsatile and non-pulsatile hemodynamic function and hemodynamic regulation, as well as finite element models of cardiac conduction processes. b) Develop and evaluate new noninvasive diagnostic methods for assessing cardiovascular function applicable to assessment of the effects of space flight on the cardiovascular system. c) Conduct animal and human studies to test hypotheses regarding the effects of space flight on cardiovascular function. d) Develop 'system identification' techniques in which physiologic data is processed to produce 'minimal models' which characterize an individual's state of cardiovascular regulation at the time the data were collected.

The research plan involves development of a hierarchy of increasingly complicated cardiovascular models, designed so that they are upwardly compatible and so that a model with an appropriate level of detail may be selected by a researcher to fit a particular modeling need. Then, these models will be complemented by the development of new noninvasive diagnostic technologies for assessing cardiovascular function. Thus, experimental data on individuals will be obtained that precisely fit within the bounds of the class of simulation models developed, allowing the models to reflect single subject similarities and differences. Finally, such concepts will be tested experimentally to: 1) validate the models and test the models' predictions; 2) test and validate the now noninvasive technologies for the assessment of cardiovascular function; and 3) to examine the effects of some of the stresses normally associated with space flight on the cardiovascular system.

The training program will involve two postdoctoral fellowships. These fellowship slots will be used to train individuals with backgrounds in physiology or the quantitative sciences to become independent investigators in the application of quantitative methods to understanding the effects of space flight on cardiovascular physiology and on the development of countermeasures to potentially deleterious consequences of space flight.

This past year has been a very productive year for the NASA Center for Quantitative Physiology, Modeling and Data Analysis. The Center received its first funding in the Spring of 1994, and initial efforts were directed to appointing staff, fellows and initiating research projects and training programs. Approximately six months later in November 17, 1994 we held our first annual symposium to report on our progress. A video tape presentation of that symposium was delivered to the NASA Life Sciences Office.

The Center is devoted to the application of quantitative mathematical methods to both modeling the cardiovascular system and to analyzing cardiovascular data so as to better understand principles of cardiovascular physiology and specifically to better understand effects of space flight on the cardiovascular system. Already a number of spin-offs of the technology developed at the Center are also being used to solve problems in clinical medicine.

Forward modeling involves the representation of a physiological system by a mathematical representation. The Center is involved in a number of projects involving forward modeling which range from the molecular level to the level of the whole organ system. These projects have focused on cardiac electrical function. In order to understand the effects of microgravity on cardiac electrical function and the generation of heart rhythm disturbances, one must understand the physiological mechanisms underlying cardiac electrical conduction properties. One project which has been led by Dr. Yuri Chernyak involves a statistical mechanical analysis of the behavior of voltage-gated channels in cardiac cell membranes. This elegant treatment of the switching behavior of the channels which control the propagation of electrical impulses in the cardiac tissue has just been published as the lead article in the December 1995 issue of IEEE Transactions on Biomedical Engineering. A second effort in the area of forward modeling has involved the development of a quantitative theory for understanding the physical principles governing propagation of the cardiac impulse on a more macroscopic scale. This effort has been conducted collaboratively by Dr. Yuri Chernyak, two graduate students Andrew Feldman and Paul Belk, and an undergraduate research assistant Linda Rosenband. This work involves representing the wave propagation relations as reaction diffusion equations and relating the coefficients to properties of cardiac muscle. This work for example has demonstrated the dependence of wave speed on curvature and other properties that are important in the development of heart rhythm disturbances. The work has also demonstrated that some of the instabilities that other investigators have identified in computer simulations may be artifactually related to the regularity of the lattice used in the simulations. We have shown how to eliminate this artifact by using a randomized lattice in the simulations. Based on these analyses physiologically realistic computer simulations of cardiac conduction and arrhythmia generation, as well as defibrillation, have been developed. A series of papers have been published describing this work. Based on the defibrillation simulations a new technique for defibrillation involving alterations in pulse shape and electrode configuration has been developed which reduces the energy required for defibrillation by a factor of approximately 3 from standard techniques. We have confirmed these predictions in a pilot set of experiments in our laboratory. This work may lead to a new generation of light weight defibrillators with a long battery life time which would be suitable for use in space as well as for civilian use.

The Center's approach to data analysis is that of Inverse Modeling where the physiological data is quantitatively analyzed in the context of a simple quantitative physiological model. We have successfully applied a series of minimal models to the analysis of physiological data. These minimal models contain only as many variables as can be reliably identified from the physiological data.

We have made great progress this year in further developing and applying auto-regressive moving average models to analysis of second-to-second fluctuations in hemodynamic and respiratory physiological signals such as heart rate, arterial blood pressure, cardiac output, and respiratory activity. We have papers published or accepted for publication on the application of linear and nonlinear cardiovascular system identification methods we have developed. In addition, we have completed analysis of a human pharmacologic study documenting the ability of the system identification method to quantify changes in autonomic activity. We have also completed a study of approximately 80

patients that demonstrate the ability of system identification techniques to detect autonomic dysfunction in patients with diabetes. The system identification technique was able to detect autonomic dysfunction even in those patients with very mild diabetes who tested normal with standard clinical tests of autonomic function. We are currently conducting a study in which we noninvasively measure cardiac output with an ultrasound technique in addition to measuring arterial blood pressure, heart rate, and respiration. Measuring cardiac output will enable us to identify for the first time the resistance baroreflex in man. These studies use tilt to simulate the effects of microgravity and gravity.

Since the signals we use for cardiovascular system identification can be acquired noninvasively, cardiovascular system identification can be applied in space. The ground based studies we have conducted demonstrate that indeed cardiovascular system identification is a sensitive and quantitative means of assessing changes in closed-loop cardiovascular regulation and thus may provide an optimal means for assessing the effects of prolonged exposure to micro-gravity and for medical diagnosis in space. These techniques also have important spin-off applications to clinical medicine on earth.

In a series of animal and human studies we have determined that micro-volt level alternation in T wave morphology provides a highly accurate predictor of susceptibility to sudden cardiac death and sustained ventricular arrhythmias. MIT has licensed this technology to Cambridge Heart, Inc. who has developed instrumentation for measuring micro-volt level electrical alternans during exercise stress. Preliminary clinical evaluations of this noninvasive method have shown it to be have an accuracy of 95% in predicting the outcome of provocative electrophysiological testing - an expensive, highly invasive, and risk bearing procedure. Meanwhile, studies conducted at MIT have shown that micro-volt level alternans is a far superior predictor of sudden cardiac death and arrhythmic events than other noninvasive predictors - signal averaged electrocardiography and QT dispersion measurements.

In other studies we have explored other approaches to measuring cardiac electrical stability. In one such study we showed that analysis of long term fluctuations (on a time scale of hours) in heart rate can identify individuals at risk for sudden cardiac death after myocardial infarction. Other studies have focused on predicting specific episodes of non-sustained ventricular tachycardia and ventricular premature beats.

Our work demonstrates that analysis of T wave alternans, long term heart rate variability, and other noninvasive measures may provide an effective means of monitoring susceptibility to ventricular arrhythmias. This technique could be readily applied in space to measure the effect of prolonged exposure to micro-gravity on cardiac electrical stability and to medical diagnosis in space and on the ground.

We have developed a technique of noninvasively imaging cardiac electrical activity by mapping the surface Laplacian of the body surface potential on the thorax. We have completed analysis of a series of animal studies to demonstrate the ability of this methodology to localize cardiac ischemia and to map cardiac arrhythmias. We are also developing inverse moving dipole methods to localize cardiac arrhythmias. These noninvasive technologies have obvious applications in space to noninvasively measuring the effects of microgravity on myocardial perfusion and alterations in cardiac conduction processes.

Five graduate students, five postdoctoral fellows, and one research staff member are engaged full time in the activities of the Center. In addition, undergraduates, Vivian Jung (Wellesley) and Linda Rosenband (MIT) are devoting major efforts conducting their senior thesis research in the Center. The Center has assisted in obtaining independent funding for some of the trainees so as to expand the activities of the Center beyond its own resources. Professor Cohen has taken over directorship of the Cardiovascular Course for the Harvard-MIT Division of Health Sciences and Technology and has introduced many modeling concepts and the use of computer simulations into this course taken by all MD students and most PhD students in the Division. In addition Professor Cohen runs a summer course for engineers and scientists from industry, government, and academia on the cardiovascular system which is heavily

oriented towards quantitative modeling approaches. Also, the Center sponsored its first annual symposium, on November 17, 1994.

The work supported by this grant has led to the development of a number of new techniques with direct benefit to clinical medicine as described in the Task Progress:

1) Noninvasive identification of individuals at risk for sudden cardiac death and ventricular arrhythmias

2) Noninvasive assessment of cardiovascular hemodynamic regulation

3) Noninvasive imaging of cardiac electrical activity

Publications, Presentations, and Other Accomplishments:

Bigger, J.T., R.C. Steinman, L.M. Rolnitzky, J.L. Fleiss, P. Albrecht, R.J. Cohen. "Power Law Behavior of RR Interval Variability in Healthy Middle-Aged Persons, Patients with Recent Acute Myocardial Infarction, and Patients with Heart Transplants." Circulation, (In press).

Chernyak, Y.B., "A Universal Steady State I-V Relationship for Membrane Current." IEEE Transactions on Biomedical Engineering, 42, 1145-1157, 1995.

Chon, K. and R.J. Cohen. "Linear and Nonlinear ARMA Model Parameter Estimation Using an Artificial Neural Network." IEEE Transactions on Biomedical Engineering, (In press).

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Feldman, A.B., J. Yin, B.E.H. Saxberg, Y.B. Chernyak and R.J. Cohen. "Vortex Wave Stability in Homogeneous Excitable Media: Simulations on a Randomized Discrete Lattice." Annual International Conference of IEEE Engineering in Medicine and Biology Society, 17, 25-26, 1995.

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Feldman, A.B., Y.B. Chernyak and R.J. Cohen. "Relations Between Continuous and Discrete Models of Excitable Media." Annual International Conference of IEEE Engineering in Medicine and Biology Society, 17, 47-48, 1995.

He B., Y.B. Chernyak, R.J. Cohen. "An Equivalent Body Surface Charge Model Representing Three-Dimensional Bioelectrical Activity." IEEE Transactions on Biomedical Engineering, 42, 637-646, 1995.

Mukkamala, R., J.M. Mathias, T.J. Mullen, R.J. Cohen and R. Freeman. "System Identification of Closed-Loop Cardiovascular Control Mechanisms: II." Diabetic Autonomic Neuropathy. (Submited).

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Osaka, M., H. Saitoh, N. Sasabe, H. Atarashi, T. Katoh, H. Hayakawa, R.J. Cohen. "Changes in Autonomic Activity Preceding Onset of Non-sustained Ventricular Tachycardia." Annals of Noninvasive Electrocardiology, (In press).

Osaka, M., K. Chon and R.J. Cohen. "Distinguishing Cardiac Randomness from Chaos." Journal of Cardiovascular Electrophysiology, 6, 441-442, 1995.

Osaka, M., S. Hirokazu, S. Tomoko, M. Hirokazu and R.J. Cohen. "Mutual Information Analysis of Temporal Patterns of Occurrence of Ventricular Premature Beats." Annual International Conference of IEEE Engineering in Medicine and Biology Society, 17, 177-178, 1995.

Raeder E.A., P. Albrecht, M. Perrott and R.J. Cohen. "Kinetics of Cycle Length Dependence of Ventricular Repolarization: Effect of Autonomic Blockade." Journal of Cardiovascular Electrophysiology, 6, 163-169, 1995.

Rosenbaum, D.S., P. Albrecht and R.J. Cohen. "Predicting Sudden Cardiac Death from T Wave Alternans of the Surface Electrocardiogram: Promise and Pitfalls." (Submtted).

Triedman, J.K., M.H. Perrott, R.J. Cohen, and J.P. Saul. " Respiratory Sinus Arrhythmia: Time Domain Characterization Using Autoregressive Moving Average Analysis." American Journal of Physiology, 268, H2232-H2238, 1995.

Environmental Biomedical Research Data Center

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Students Funded Under Research: 5

Task Description:

The Environmental Biomedical Research Data Center is a system of analytic predictive modeling of environmental stress-responses, basic and applied research records data bases, operational life-support data bases, environmental biomedical bibliographic sources, 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.

Examples of present activity and specific aims of the proposal include incorporation of expensive new data concerning physiologic and toxic effects of oxygen on human organ systems; the predictive modeling of this oxygen tolerance information; 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 adaptations to hypoxia and hypercarbia; and the interactions of respiratory environmental stresses and respiratory function in graded degrees of physical work. 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 existing assets of the Environmental Biomedical Research Data Center have evolved systematically over the past 25 years. Initial data inclusions and analysis consisted of mathematical integration of new and existing information to aid concept and further research planning. Work was performed by simple calculator, diagramming and hard copy organized storage. The present data accumulation, recording, storage, and analyses procedures are computer-based, with hard copy back-up. Accomplishment during the past year has included establishment and analyses of a large data base for Doppler monitored Venous Gas Embolism (VGE) in existing laboratory hyperbaric decompression trials. Specific new analytic methods have shown correlation of the empirically monitored Doppler VGE index and a theoretical index of gas phase evolution in hyperbaric decompression. The question raised is whether refinements in analyzing degrees of decompression stress in terms of venous gas embolism can also be used to validate new models of hypobaric decompression stress being developed by NASA Johnson Space Center.

Separate analyses of human dynamic responses to abrupt inspiratory hypoxia, and hypoxia with use of CO_2 to prevent hypocapnia, have provided data on the time courses of induced changes of interrelated factors in regulation of respiration, blood gas composition, brain blood flow and brain oxygenation. These patterns of dynamic relationship raise the question and opportunity of establishing quantitative predictive models of adaptation to varied situations of altered external atmosphere, and the interplay of chemical factors in respiratory and brain circulatory regulation.

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 the basic experiment data 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, gas toxicity, including carbon monoxide poisoning, oxygen tolerance and poisoning, physical work in hypoxic atmospheres, and adaptation to increased atmospheric carbon dioxide.

These broad opportunities provide for determining degrees and limits of human physiological capabilities as these result to normal working and to extreme emergencies. They bridge the scope of normal human endeavor for the common man 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.

Publications, Presentations, and Other Accomplishments:

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Clark, J. M., C. J. Lambertsen, R. Gelfand, and E. J. Hopkin "Analysis of factors that determine rates of recovery from human pulmonary oxygen poisoning in Predictive Studies V. Abstract." Undersea Hyperbaric Med., Submitted, 1995.

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Clark, J. M., R. Gelfand, C. J. Lambertsen, W. C. Stevens, G. Beck, Jr., and D. G. Fisher "Human tolerance and physiological responses to exercise while breathing oxygen at 2.0 ATA." Aviat. Space Environ. Med., 66, 36-345, 1995.

Clark, J. M, R. Gelfand, G. Beck, Jr., B. A. Youdelman, E. J. Hopkin, and C. J. Lambertsen "Effects of head-out and total immersion at 1.0 ATA on ventilatory and cerebral circulatory responses to progressive hypercapnia. Abstract." Undersea Hyperbaric Med., 21 (Supp.), 36, 1994.

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Gelfand, R., C. J. Lambertsen, G. Beck, Jr., and J. M. Clark "Dynamic responses of SaO_2 and "CBF" to abrupt exposure to inhaled 10% O_2 / 4% CO_2 at rest, followed by 50 and 100 watts exercise." Abstract. Undersea Hyperbaric Med., 22 (Supp.), 70, 1995.

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Development of Data-Driven Models to Describe Astronaut Performance in Microgravity: Full-Body Dynamics and Control

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Students Funded Under Research: 5

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 O G dynamics, a detailed control model will provide the appropriate physiological performance of astronaut whole body motion, especially during impact. The Shuttle era has demonstrated numerous successful capabilities ranging from deployment of satellites, material science and life science experiments, and 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 proposed effort is immediately applicable to Shuttle/Mir missions, and the 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 a demonstration of the Intelsat VI (mis)capture. In this initial simulation, the satellite dynamics, astronaut kinematic arm motions, and 3-D animation were presented. The demonstration displayed the utility of the analytical techniques and properly modeled the spiral nutation of the satellite after the first attempted capture. Future experimental studies will include motion analysis and muscle activation levels on a partial gravity simulator to assist in the development of the astronaut multsegment model and space suit mode. The research effort will yield an integration of astronaut dynamic motion and control strategies that will be displayed as 3-D animations.

Simulation of astronaut motions during extravehicular activity (EVA) tasks was performed using computational multibody dynamics methods. The application of computational dynamic simulation to EVA was prompted by the realization that physical microgravity simulators have inherent limitations: viscosity in neutral buoyancy tanks; friction in air bearing floors; short duration for parabolic aircraft; and inertia and friction in suspension mechanisms. These limitations can mask critical dynamic effects that later cause problems during actual EVAs performed in space.

Methods of formulating dynamic equations of motion for multibody systems are implemented with emphasis on Kane's method, which forms the basis of the computer analysis. Formulation of the equations of motion for a two degree of freedom arm was presented as an explicit example. The four basic steps in creating the computational simulations were: system description, in which the geometry, mass properties, and interconnection of system bodies are input to the computer; equation formulation based on the system description; inverse kinematics, in which the angles, velocities, and accelerations of joints are calculated for prescribed motion of the endpoint (hand) of the arm; and inverse dynamics, in which joint torques are calculated for a prescribed motion. A graphical animation and data plotting program, EVADS (EVA Dynamics Simulation), was developed and used to analyze the results of the simulations that were performed on a Silicon Graphics Indigo2 computer.

EVA tasks involving manipulation of the Spartan free flying astronomy payload, as performed during Space Shuttle mission STS-63 (February 1995), served as the subject for two dynamic simulations. An EVA crew member was modeled as a seven segment system with an eighth segment representing the massive payload attached to the hand. For both simulations, the initial configuration of the lower body (trunk, upper leg, and lower leg) was a neutral microgravity posture. In the first simulation, the payload was manipulated around a circular trajectory of 15 cm radius in 10 seconds. It was found that the wrist joint theoretically exceeded its ulnal deviation limit by as much as 49.8° and was required to exert torques as high as 26 N-m to accomplish the task, well in excess of the wrist physiological limit of 12 N-m. The largest torque in the first simulation, 52 N-m, occurred in the ankle joint. To avoid these problems, the second simulation placed the arm in a more comfortable initial position and the radius and speed of the circular trajectory were reduced by half. As a result, the joint angles and torques were reduced to values well within their physiological limits. In particular, the maximum wrist torque for the second simulation was only 3 N-m and the maximum ankle torque was only 6 N-m.

The new questions being addressed are the effect of the space suit on performance; increasing the degrees of freedom in the human model to provide more realistic simulations; and are there experimental data to use to verify the simulations or will an experimental study be necessary?

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 and vestibular patients). The analysis package and computer simulations provide dynamic analysis as well as computer animation. 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 package could be easily modified to be useful for 1 g, everyday concerns such as, work injury analysis, clinical analysis, and real time computer animations of most motions.

Publications, Presentations, and Other Accomplishments:

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Newman, D.J. "(Abstract) Human Posture Modeling: An Assessment of Altered Gravity Adaptation." Annals of Biomedical Engineering, Vol 23, supp. 1, S89, 426, 1995.

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Newman, D.J. "Human Posture Modeling: An Assessment of Altered Gravity Adaptation." Biomedical Engineering Society Annual Fall Meeting, Boston, MA, October 7, 1995.

Newman, D.J. "Astronaut Adaptation of Performance in Altered Gravity." Space Grant Symposium, UMDNJ, New Jersey's University of the Health Sciences School of Osteopathic Medicine, November, 1995.

Newman, D.J. "Engineering Analysis of Astronaut Adaptation in Altered Gravity." MIT, Department of Aeronautics and Astronautics, Faculty Meeting, November 1995.

Spacelab Rotating Chair Analysis

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Funding:

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Students Funded Under Research: 2

Task Description:

Over the past decade, we have conducted rotating chair angular vestibulo-ocular reflex tests on five different Shuttle/Spacelab missions using a consistent test procedure (SL-1: Oman and Kulbaski, 1987, 1988.; Oman et al, 1988, and Liefeld, 1993; D-1: Oman and Weigl, 1989, 1990; SLS-1: Oman and Balkwill, 1993; Young, et al, 1993; IML-1: Oman and Calkins, 1993; SLS-2: Oman and Pouliot, in preparation). Each mission yielded eye movement from 4-5 subjects. The data sets from each mission were analyzed separately, using several different methods. The goal of the proposed one year extended data analysis project is to combine all 5 data sets (n=21 pre/post, n=12 inflight), plus data from 3 astronaut control subjects. We want to analyze this larger population data set from a fresh perspective, using a common analysis methodology, and ask questions which cannot easily be answered from small population data sets. Do consistent changes in the human vestibulo-ocular reflex (VOR) parameters occur among astronauts which could account for reports of space sickness and oscillopsia? Do the responses of subjects who experienced significant space sickness inflight (n=13) differ systematically from those who did not (n=8)? Our ability to discriminate statistically significant changes in gain and time constant was compromised due to the small "n" on each flight, the inherent variability of human responses, and VOR dropouts in fatigued crew members. Comparison results from different missions has been difficult, because several different nystagmus analysis techniques were used. During the past two years, we have improved our methods for calculating nystagmus slow phase velocity, detecting and removing VOR dropouts, and fitting the data to mathematical models. Using these techniques, we analyzed data from the IML-1 mission and found a statistically significant inflight increase in inflight and postflight VOR gain, a corresponding decrease in VOR time constant, and a rank correlation with space sickness intensity. We have subsequently reanalyzed pre/postflight data from the SL-1 mission using our newer methods, and also found a statistically postflight gain change that correlated with previous inflight sickness intensity. Analysis of data from the recent SLS-2 mission is being accomplished using these newer methods.

Analysis of horizontal angular VOR time constants obtained in flight on SLS-1, SLS-2 and IML-1 Spacelab missions indicate a statistically significant relationship between time constant changes in weightlessness and previous space flight experience. On average, subjects who had flown previously

on one or more shuttle flights show a persisting loss of angular VOR velocity storage in flight, while crew members making their first flight show a recovery of vestibular velocity storage, and time constant values equalling or exceeding those seen preflight. This result suggests that while all astronauts discount vestibular cues upon initial exposure to weightlessness, rookies subsequently show evidence of adaptation, while veterans continue to discount vestibular inputs - at least through the first week in orbit. This has intriguing implications relative to astronaut training and preadaptation. A paper describing these results has been accepted for publication in the Journal of Applied Physiology. Current efforts are aimed at an integrated reanalysis of SLS-1 and SLS-2 data using a common methodology.

The goal of this study is to better understand the influence of gravity cues on the human vestibulo-ocular reflex (VOR). The vertebrate nervous system evolved in an environment where the stimulus to the body's 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 to orientation, and thus to better understand the role of gravity in the fundamental sensory, motor, and cognitive mechanisms which normally subserve spatial orientation on earth. Vestibulo-ocular reflex mechanisms allow us to stand and move about actively in the environment, maintaining our sense of direction and the stability of our visual world. We only become aware of these functions when they are compromised by inner ear or central nervous system disease. Unfortunately, more than 90 million Americans suffer from some type of balance disorder. Patients with VOR 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. Our preflight studies of the VOR in astronauts has provided potentially clinically important data on the test-retest repeatability of VOR gain and time constant parameters in 1-G, and our inflight data have furnished new information on how the human VOR adapts to an altered gravitoinertial environment.

Biophysical, Mathematical Models of Gas Phase Formation

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Funding:

Project Identification: 199-70-31-20

Solicitation: 93-OLMSA-07

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Expiration: 1/98

FY 1995 Funding: $74,234

Students Funded Under Research: 0

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 in to 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.; this model 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; this model 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 data base. These models and their parameters will be evaluated by increasing their ability to predict the occurrence of both decompression sickness (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.

Dr. Wayne Gerth

This model has now been modified to accommodate gas bubbles present in the venous return (Doppler-detectable gas bubbles) and their effect on gas transport from the tissue. This has incorporated the movement of gas bubbles from tissue to blood. The incorporation of survival models as contrasted with pure logistical ones allows the inclusion of time at altitude.

The model shows a dependence of gas bubble presence on the results of a decompression whereby they function as an augmentor and dissolved inert gas is removed from tissue by the two phase system. This is predicted by the model to be beneficial if decompression sickness does not first appear. The model shows an independence of the risk of decompression sickness with oxygen prebreathe at altitudes of 30,000 feet.

The model is also being modified for use with the problem of flying in aircraft after exposure to pressure (i.e., flying after diving). This problem arises at NASA when astronauts train in the WETF and then are required to fly to another destination within a minimum duration after exposed to pressure.

Dr. Michael Gernhardt and Dr. Michael Powell

The model has been modified such that it now has menu options for a time- and size-dependent diffusion barrier. Into the model has been the incorporation of stress-assisted nucleation, a concept that appears to be a major factor for decompression in null gravity bubble enhancement using tribonucleation equation with a user specified frequency. This is an interesting addition which is being pursued and is being considered for combination with the variable diffusion barrier. By the use of these ideas, we believe we can place some realistic limits on the parameter values just with boundary conditions alone.

Relationships between this model and that of Van Liew and Burkhard are currently being explored. Modification include the stochastic addition of nucleation/growth sites both prior to and during the depressurization. Analysis of gas washout with exercise has indicated that mild exercise is beneficial, and correlates well with the "effective" tissue halftime.

The development of decompression tables and particularly a rational understanding of the mechanisms behind decompression sickness would be of value for both SCUBA and commercial divers.

Modeling of Cardiovascular Response to Weightlessness

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Project Identification: 199-70-37-20 Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95 Expiration: 1/98

FY 1995 Funding: $129,559 Students Funded Under Research: 7

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 will be studied by developing a lumped parameter 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 will be 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. The plan for year 1 encompasses initial development of the numerical model of the systemic circulation including short-term effects of hydrostatic pressure in the ventricle and vasculature and thorax/abdomen structural effects on atrial and ventricular transmural pressure. For year 2, pulmonary circulation will be added and in year 3 intermediate and long-term effects of fluid volume adjustment, extravascular fluid shifts, spinal extension and some aspects of neurohumoral control will be added. The sophistication of the numerical model will precede that of the hydraulic simulator (which will incorporate only short-term responses because of the limited duration of 0-G aboard the KC-135) so that computer results can be used to guide the construction of 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.

A new physical model of the human systemic circulation has been built for studying the effects of gravity on the cardiovascular system. The model incorporates an artificial heart, a caudal venous pool and three vascular sections - one for the head and arms, one for the torso and one for the legs and feet - to allow investigation of fluid shifting in the vasculature as well as changes in cardiac performance due to gravity. The improved ventricular model consists of a flexing, polymer sac inside a pressurization chamber. Ultrasonic crystals for the measurement of ventricular diameter are incorporated into the walls of the ventricular sac. Its configuration and pulsatile function approximates the anatomy, endocardial wall movement and pressure-volume relationship of the natural left ventricle. The new venous pool chamber consists of a flaccid, polymer sac inside a rigid, air chamber. Its nonlinear response is similar to that reported for natural veins. Each vascular section has four elements - arterial resistor, arterial compliance, peripheral resistor and peripheral compliance. A small amount of inertance is also introduced in connecting tubing. The resistors are made from a porous plastic sheet over which a motorized plate slides for adjustment of the resistance value. Each compliance unit incorporates a coil spring-loaded piston moving inside a cylinder sealed with an elastomeric diaphragm. The compliance values are adjustable by changing the spring constant. For the first year experiments, the control strategy for investigating a range of operating conditions on the cardiac function (flow vs. pressure) curve was to manually change the three peripheral resistance values and have the controller automatically adjust circulating fluid volume to maintain 95 mmHg average aortic pressure. This strategy, while somewhat different from the physiologic system, is easier to implement than automatic control of three resistance values while at the same time keeping the flow distribution among the vascular sections constant. The resulting data, however, is uncompromised and is the same regardless of which parameter is automatically controlled. The space frame containing the experiment is attached to the base plate with hinges so that the frame can be tilted down to easily change the experimental posture from upright (standing) to supine. In the supine position, an additional subframe is attached to raise the caudal vascular section and the caudal venous pool to simulate the launch position. The experiment is monitored with four flow probes, ten pressure transducers, a pair of ultrasonic crystals and one accelerometer. Very physiologic-looking pressure and flow conditions were created. In-flight measurements made with the hydraulic model in the supine position confirmed the presence of a hydrostatic pressure difference in the ventricle. Compared to the preceding 1.8-G pull-up period atrial pressures always increased approximately 4 mmHg with entry into the weightless period. However, for greater preload conditions, when comparing to the preceding 18-G pull-up period, the stroke volume decreased 14% with entry into weightlessness, but for the lower preload conditions, the stroke volume increased 12%. The explanation for this difference in stroke volume response is currently being explored. During 1-G experiments, we succeeded in simulating the acute cardiovascular response to weightlessness reported from the SLS-1 and SLS-2 missions where an increase in stroke volume and ventricular end-diastolic dimension was found in the presence of reduced filling pressure. We have proposed that this paradoxical finding is due to a reduction in the intrapleural pressure. To simulate this condition, a 3 mm Hg vacuum was applied to the ventricular chamber during iastole. An extensive computer program was developed to simulate the systemic arterial system. The computer model, which incorporates over 1000 elements (lumped resistance, compliance and inertance), was used to choose values of the elements in the experiment that will most closely approximate the human system. The original plan for the second year was to add the effects of transmural pressure on the atrium and ventricle to the hydraulic model and to extend the computer model to the pulmonary circulation. The revised plan will be the same with the following exceptions: 1) Ventricular transmural pressure was already incorporated in year 1, so only atrial transmural pressure will need to be added in year 2. 2) The computer model of the systemic arteries developed during year 1 is much more extensive than proposed, but does not include the veins and ventricle or hydrostatic or transmural pressure. We plan to add these effects as well as the pulmonary circulation during 1996. We expect also to include during year 2 some effects of neurohumoral control that were not scheduled to be

included until 1997. 3) Because of mechanical problems with the KC-135, only one of the scheduled four flight days was logged in 1995. We plan to make up the missed flight days early in 1996 and to also fly further experiments as originally scheduled later in year 2.

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 9 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 precluded 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.

Publications, Presentations, and Other Accomplishments:

Pantalos, G.M., Sharp, M.K., O'Leary, D.S., and Gillars, K.J. "Simulation of cardiovascular adaptation to weightlessness." American Society of Gravitational and Space Biology Conference, Washington, DC, October 26-28, 1995.

Applications of Mathematical Models in the Study of Countermeasures to Cardiovascular Deconditioning

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Funding:

Project Identification: 199-70-31-13 Solicitation:

Initial Funding Date: 10/92 Expiration: 9/95

FY 1995 Funding: $120,000 Students Funded Under Research: 0

Task Description:

The purpose of the proposed study is to provide continuing computer simulation support to the cardiovascular research program at NASA Johnson Space Center. The study addresses development and application of mathematical models of circulatory and cardiovascular systems to evaluate the effectiveness of specific countermeasures to orthostatic intolerance that have been proposed for experimental investigation. Model development aims to build on the improvements made in a previous study funded by NASA. Specific model revisions are proposed as identified from the previous study. The applications involve computer simulation using the models to predict the circulatory response with various proposed countermeasures. The simulations are also aimed at aiding the analysis and interpretation of results from space flight and ground-based experiments through comparison of simulated and experimental data.

The principal models involved are the Guyton model of circulation and a pulsatile model of the cardiovascular system. The Guyton model has been modified for specific simulations and the pulsatile cardiovascular model has been simplified to minimize parameter uncertainties. The proposed study will continue the revision of these models to enhance their utilization in space flight cardiovascular research. The specific tasks proposed constitute the logical follow–up on the development of modeling and computer simulation as a supportive element of NASA's cardiovascular research program.

The project involves two different mathematical models – a simple model of the cardiovascular system and the Guyton model of circulation. Tasks related to the cardiovascular model performed during FY95 include:

(1) Improvement of the model with incorporation of a nonlinear pressure–volume relationship for the upper body compartment and a number of modifications related to implementation of the model in C language. The improved model was used to study the differences in cardiovascular responses to standing and lower body negative pressure (LBNP) and the changes in these responses caused by exposure to weightlessness. Appropriate changes in model parameters were made, which reflected plausible hypotheses to distinguish standing from LBNP. The simulated responses were compared with available data from astronauts obtained during preflight and postflight tests. The results of the study were presented at the Biomedical Engineering Society Meeting held October 1995 in Boston, MA, and also

at the Fourteenth Annual Houston Conference on Biomedical Engineering Research held February 1996 in Houston, TX.

(2) Discussion of the model and simulation results with the colleagues and students of Prof. Richard Cohen, Director of NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis at the Massachusetts Institute of Technology, Cambridge, MA. The discussion explored areas of model improvement as well as the application of system identification techniques to fit the model to data from individual subjects (with the objective of estimating changes in 'internal' parameters of the system and thus developing indices to assess cardiovascular status).

(3) Preparation of a manuscript detailing the model development and the simulation results for publication in a journal. The manuscript is currently being revised per reviewers' comments.

(4) Review of the following book for the Annals of Biomedical Engineering: The Physics of Heart and Circulation, Edited by J. Strackee and N. Westerhof, Institute of Physics Publishing, Bristok, UK, and Philadelphia, PA, 1993

In the area of the Guyton model of circulation, simulations were preformed using the November 1992 version of the model, modified to simulate hypogravic conditions during FY93 and FY94 of the project period. Tasks completed include:

(1) Initialization of the model in the standing position, with a tilt angle of 900 instead of the normal 00 corresponding to the supine posture. The model produced stable conditions in the standing position, but did not restore the supine initial conditions when switched from 90 to 00 degree (because of the existence of a number of stable equilibrium points due to nonlinearities present in the model).

(2) Simulation of long–term weightlessness using head–down tilt as analog. The model produced acceptable time–course of changes in many variables, but the changes exhibited mild oscillations before reaching steady state. The model needs to be tested further with comparison of simulated and experimental data in order to ensure its capability to simulate hypogravic conditions accurately. Recent data from the fluid–electrolyte studies conducted in SLS–1 and SLS–2 missions will be extremely helpful in validating the model. A final report detailing the model modifications made and software items developed is under preparation.

(3) Completion of a book chapter on applications of mathematical modeling and computer simulation in space flight biomedical research (to be published in Space Biology and Medicine, Volume III: Humans in Space flight, Book 2: Effects of Other Space flight Factors).

The models employed in this project are of the mechanistic type, i.e., the relationships among the physiologic variables are derived mostly from mechanistic considerations. Although the models were developed for analyses of data related to space flight studies, they have much wider applicability.

The Guyton model of circulation is the most comprehensive model of fluid and electrolyte regulation available to date. It is a valuable tool in the conduct of experimental studies, and is used by many investigators around the world. It is also used as a teaching aid. But the model is by no means complete, and is being continually improved (in the light of additional knowledge about the mechanisms involved). The modifications of the Guyton model made in this project contribute to the evolutionary development of the model with enhanced capability and wider applicability.

The simple cardiovascular model developed in this project, when coupled with suitable identification techniques, will be useful to realistically assess the 'internal' changes in cardiovascular function under the influence of external stimuli. Such an approach has been used, but is not widely followed because of lack of an acceptable model. The modeling approach used here can lead to an acceptable model, if

developed step–by–step with inclusion of (1) right ventricle (2) pulmonary circulation (3) separate sympathetic and parasympathetic control elements.

Publications, Presentations, and Other Accomplishments:

Karam, E.H., Srinivasan, R.S., and Charles, J.B. "Simulation of cardiovascular response to lower body negative pressure up to presyncopal levels." Math. Modeling and Scientific Computing, vol. 4, 327-332, 1994.

Karam, E.HJ., Srinivasan, R.S., Charles, J.B., and Fortney, S.M. "The effect of blood volume loss on cardiovascular response to lower body negative pressure using a mathematical model." Proceedings of the 15th Ann. Internat. Gravit. Physiol. Meeting, Barcelona, Spain, October 3-8, 1994.

Melçhior, F.M., Srinivasan, R.S., Thullier, P.H., and Clère, J-M. "Simulation of cardiovascular response to lower body negative pressure from 0 to -40 mmHg." J. Appl. Physiol., vol. 77, 630–640, 1994.

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Simanonok, K.E., Srinivasan, R.S., Myrick E.E., Blomkalns A.L., and Charles J.B. "A comprehensive Guyton model analysis of physiologic response to preadaptation of blood volume as a countermeasure to fluid shifts." J. Clin. Pharmacol., vol. 24, 440-453, 1994.

Srinivasan, R.S., Leonard, J.I., and Charles, J.B. "Application of the Guyton model of circulation in the study of space flight circulatory changes." Proceedings of the International Federation of Automatic Control Symposium on Modeling and Control in Biomedical Systems, March 27-30, 1994, Galveston, TX.

Srinivasan, R.S, Leonard, J.I., and White R.J. "Applications of Mathematical Modeling and Computer Simulation in Spaceflight Biomedical Research. In: Foundations of Space Biology and Medicine, Joint Publication by US and Russia (to be published in Space Biology and Medicine, Volume III: Humans in Spaceflight, Book 2: Effects of Other Spaceflight Factors)."

Srinivasan, R.S., Simanonok, K.E., and Charles, J.B. "Computer simulation of the effect of dDAVP with saline loading on fluid balance after 240-hour head-down tilt." Proceedings 15th Ann. Internat. Gravt. Physiol. Meeting, Barcelona, Spain, October 3-8, 1994.

An Advanced Approach to Simultaneous Monitoring of Multiple Bacteria in Space

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Funding:

Project Identification: 199-04-17-10
Solicitation: 93-OLMSA-07

Initial Funding Date: 3/94
Expiration: 3/97

FY 1995 Funding: $302,508
Students Funded Under Research: 2

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 cm^2 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.

Progress has been made on three fronts during the last year. First, an extremely effective strategy for identifying potential RNA probe target sequences and capture sequences has been developed for the detection of an exclusion list of specific organisms (bacteria/fungal) in the space environment. Also for the probe-based microorganism identification assay, the effect of the "hybridization surface" on target / probe binding interaction provided insights into optimizing the affinity and selectivity of IGS binding. A combination of molecular modeling and oligonucleotide chemistry has been utilized to develop two new types of oligonucleotide probes for CCD detection. Finally, initial experiments utilizing the proximal CCD imaging techniques have demonstrated excellent sensitivity and specificity for the 16S rRNA hybridization arrays. Future developments include the employment of back illuminated CCDs to substantially increase the detection threshold with continued high specificity.

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 the high sensitivity and miniature format.

Publications, Presentations, and Other Accomplishments:

Eggers, M.D. "A biochip for rapid molecular detection." Biochip Array Technologies, IBC, Washington, D.C., May 10, 1995.

Eggers, M.D. "A review of microfabricated devices for gene-based diagnostics." Microfabrication Technology for Research and Diagnostics, CHI, San Francisco, CA, Sept. 28-29, 1995.

Eggers, M.D. "A biochip for rapid molecular detection." Third International Conference on Automation in Mapping and DNA Sequencing, AMS '95, Berkeley, CA, Nov. 7, 1995.

Eggers, M.D., and D. Ehrlich. "A review of microfabricated devices for gene-based diagnostics." Hematologic Pathology, Vol. 9, No. 1, 1995.

Patent In Process, U.S. Patent #: Submitted November 16, 1995. Hogan, M., T. Powdrill, A. Mallik, B.Iverson, N. Akiyama, D. Xiao "Integrated nucleic acid hybridization devices based upon active surface chemistry."

Advancement in Determining Hazardous Volatile Organic Compounds in Air

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-04-17-13

Solicitation: 93-OLMSA-07

Initial Funding Date: 3/95

Expiration: 3/98

FY 1995 Funding: $145,658

Students Funded Under Research: 0

Task Description:

During the last three 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 that may arise 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 state. These items center largely on the artificial intelligence of handling analyzer spectra and 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 data bases, and predictive capabilities for unknown or unprogrammed vapors through an improved understanding of the foundations of response.

Ion mobility spectrometry (IMS) has been successfully used to monitor or detect hazardous organic compounds for over a decade in niche applications and IMS is scheduled for use on International Space Station (ISS) for air quality monitoring. During the last year, the principles and predictive tools for this analyzer have been advanced through efforts of this program beyond the nominal understanding considered heretofore acceptable for specialized uses of IMS. The importance and need for these advances reside in the creation of automated intelligence (to minimize the need for human resources) and in the formation of tools for the identification of chemicals not previously characterized by IMS. The experiments completed in FY95 support a model where IMS spectra arise from considerations both of proton affinities in the initial event of ion creation and of ion stabilities in the non-equilibrium, uni-polar drift region. The project is ahead of schedule for certain developments in hardware and software and on-schedule in other areas. The creation of a data base, under conditions similar to that for ion mobility spectrometers to be used on-board ISS, was inverted with hardware development and is out of sequence but within the overall schedule and timeline.

The first major objective of this program was to discover and develop the foundations of ion mobility spectra toward automated identifications of results from analysis of atmospheres inside spacecraft.

Toward this objective and the overall goal of maturing a technology already scheduled for ISS, advances have occurred in the areas of science, instrumentation, software, and technology. Each of these will be described in separate sections below.

A pivotal development occurred in FY95 as we constructed a first comprehensive model for the creation of ion mobility spectra. This was made using several key experiments in combination with calculations from known ion molecule chemistry. Before this year, the problem of predicting mobility spectra was centered on calculating which ions could appropriate charge through atmospheric pressure chemical ionization reactions. Apparent contradictions between results from APCI-mass spectrometry and ion mobility spectrometry cause the issue to be recast in our program. The presence of ions in an IMS spectrum is now seen as the combination of two steps. In the first step, ions and ion clusters are created by proton transfers and in the second step, ions move through an electric field and against collisions with a buffer gas (i.e., an unipolar, nonequilibrium condition). In this region, only ion decompositions can occur for ion clusters in an irreversible manner. This was explored by characterizing dimer and trimer ions in IMS between ambient temperature and 20°C. We found that peak ratios between dimer ions (M2H+) and trimer ions (M3H+) were sensitive to temperature and trimer ions, with some exceptions, would not survive at temperatures above 25°C. These studies suggested that the issue of ion survival was relevant to predicting ion mobility spectra. Calculations suggested that dimethylsulfoxide should survive as a trimer ion on IMS time scales at ambient temperature and calculations were experimentally confirmed. In the last half year of the first calendar year of this project (end of FY95 and beginning of FY96), an elevated temperature ion mobility spectrometer has been built to explore APCI reactions from 25 to 300°C. These temperatures are relevant to instrumentation planned for use on ISS and studies will seek to determine the quantitative relationship in the ratio of peak heights for monomer (MH+) and dimer ions. In short, the presence of a peak in an ion mobility spectrum is linked to ion stabilities and the approach to predicting ion mobility spectra through proton affinities alone must be augmented. If these ideas are correct, then ions with greater clustering than that for dimer ions should be created in the ion source and should be observable under conditions where such ions are stabilized. Thus, the discovery of trimer ions for alcohols is a confirmation of the foundational premise of this understanding of spectral origins and has importance as a type of consistency check on this approach. Actual kinetics of decomposition can be observed by varying the flight distance for an ion. The survival of cluster ions (otherwise considered too unstable to survive the drift region) in uni-polar nonequilibrium conditions was explored in detail and the procedures to map the kinetic terms needed for predictive models were developed using sterically hindered amines. Sterically hindered amines such as triethylamine, tripropylamine and others were known not to exhibit dimer ions under ambient conditions of temperature and pressure. However, reduced temperatures allowed dimer ions in sterically hindered amines to be detected. Spectra for triethylamine at fixed concentration (ca. 30ppb) and drift length (5cm) with a variety of temperatures are shown in the Figure 1. The reactant ion peak has the shortest drift time, followed by the monomer ion and then the dimer ion. Note that at -4°C no dimer ion was apparent, however at -32°C, the dimer ion was dominant. In order to make kinetic measurements of these cluster decompositions, an IMS cell was fabricated with a variable drift length and temperature control (down to ca. -50°C). At a fixed temperature, by adjusting the drift length (i.e. changing the time the ion spends in the nonequilibrium drift region) the reverse rate constants of these clusters can be calculated. Collection of these rate constants at a variety of temperatures allowed us to form a van't Hoff plot and provide a measure of ion stability. Results have been obtained for triethylamine, diethylmethylamine, and diethlypropylamine is being studied. These findings will be used to formulate a model to allow us to quantitatively predict cluster ion peak intensities at a given temperature and distance. The apparatus for these studies are shown as photographs in Figures 2 and 3.

In a professional association and agreement to cooperate on IMS and databases, an accomplishment was made on data set interpretation by Professor Jurs' team at Penn State University (PSU) through student Matt Wessel. An existing database for ca. 220 organic chemicals from our laboratory was subjected to neural network (NN) training and the PSU team found that modeling was successful based upon monomer ions alone with some notable out-lying spectra. These spectra thus represent a list of

chemicals where ionization behavior is not ordinary and thus worthy of close inspection in the overall program of building predictive models. Nonetheless, NN methods were successful for a large number of chemicals. In associated work, started before FY95 but relevant to our project, another professional association with Dr. Snyder showed that for a finite set of volatile organic chemicals representative of space cabins, multivariate methods were suitable with ion mobility spectra. In short, several professional collaborations, deemed helpful in accomplishing our overall goal, led to success in FY95 and these will be developed and expanded in FY96. The importance of this is that our confidence in handling ion mobility spectra and in viewing such spectra as tools for identifications has grown and we are poised to make essential measurements in FY96.

A central issue in the second year of this project involved the creation of a high temperature, high speed, ion mobility spectrometer for use in gas chromatography-ion mobility spectrometer instruments. This part of the research program was accelerated and moved to the middle-of the first year when the availability of such hardware was deemed helpful in creating a refined database and in addressing moisture/temperature effects in APCI reactions (for predictive tools). As such, effort was given to formalizing proven electronics in a single control board (see Figure 4). These boards were designed at NMSU and manufactured privately. A drift tube for IMS was also created using professional grade CAD package and was made to meet both performance requirements (extended temperature range, memory effects, and speed of response) and ease of repair. Moreover, the drift tube was designed for a modular construction where new ion sources (such as a non radioactive source) can be added to replace an existing component without complications in wiring or geometries. During this year, studies were made using high-speed medium resolution gas chromatography with a previously undisclosed column design that originated in the former USSR military research program. The concept of multicapillary columns (MCC) where a bundle of extraordinarily narrow capillaries exist in a also narrow column (ca. 2 mm OD) arose in the mid 1960s but was left undeveloped in the West. However, opportunities arose for our research program to examine experimentally one of these columns. Our interests were also linked to the earth-based applications or possibilities for a GC/IMS analyzer with MCC columns (see the section on FY95 Earth Benefits). Studies were made to characterize the MCC column and reference the behavior to a standard capillary column of the kind in use worldwide. The results of these investigations showed that the MCC column had advantages in high volumetric flows and large sample mass loading (versus traditional columns) without losses in speed of analyses (<60s) and resolution. Moreover, the size (ca. 15 cm) and convenience of connections made the MCC attractive. An unknown aspect is the suitability for temperature programming and this will be explored in the next year.

A critical appraisal of IMS technology will reveal that the technology has lacked appropriate support in areas of data bases, artificial intelligence, and software. All of these were targets for this last funding cycle and an exciting discovery occurred in the software tasks. A commercially available package that allows convenient creation of complete software packages in Windows was discovered and purchased. The package, Test point for Windows, is suitable for a range of ADC boards and has been demonstrated to operate for a GC/IMS system. A version of IMS software, suitable for mouse-driven applications is planned for completion in the first 4 months of FY96.

A fundamental limitation of IMS technology has been the narrow linear range of response. An attempt was made in FY95 to extend the linear range using a servo inlet first described at the Univ. of Manchester Institute of Science and Technology (UMIST). After four months of exploration with this prospective inlet for an ion mobility spectrometer, an unfavorable conclusion was reached and the inlet (after over five modifications) was considered flawed by the high diffusivity and turbulence of gases. Nonetheless, partial success was attained with an additional 3 fold increase in the linear range (while a extension of 102 or 103 is needed. The servo approach in inlet technology will be revisited in EWY96 with fluidic logic gates in place of the UMIST design. In a minor but important development, hardware was created to extensively and inexpensively scrub air to less than 100 ppb moisture and a means of adding moisture in a controlled manner were created and installed in the laboratory. Moisture is now routinely controlled to ca. 100 ppb and higher.

The questions from last year and the answers are listed here and can be viewed in context of the discussion above.

1. What are the essential components necessary to provide predictive and interpretive tools for ion mobility spectrometry? As best as can be ascertained, a large number of possible ion products arise from APCI reactions of ordinary vapors of health and safety importance. The essential element seems to be assessing which ions survive the nonequilibrium of the drift region. Thus, the kinetics of decomposition of ions (as a measure of ion stability seems necessary). Seemingly, these terms will need to be determined or predicted to take IMS spectral processing to an advanced level, a goal of this program. In the next year, the new question from this work will be: Can we formalize a set of results on a quantitative or semiquantitative basis to include cluster stability terms with proton affinities to produce a distribution of ions that resemble those in an ion mobility spectrum?

2. Can neural networks or multivariate methods provide gain in the identification of ion mobility spectra? In a limited test set of IMS spectra(without any reference to retention times), a multivariate method showed success in recognizing ion mobility spectra. However, ca. 15 spectra of otherwise good quality (i.e. sufficient product ion intensity) failed to train for monomer ions. The APCI reaction chemistry of these molecules will be explored and are already known to represent unconventional responses; however, the tentative conclusion is that neural networks trained nicely on monomer ions. In the next year, a new data set for elevated temperature will be submitted for analysis. The new question will be: do elevated temperature spectra allow training in neural nets and will the information content be altered by the temperature?

3. What hardware and software configurations are helpful in advancing IMS technology? This question arises not from a year of study but is a culmination of over 14 years of work in IMS. At present, the hardware has been finalized with a drift tube that is pneumatically sealed, suited for elevated temperature, and built from metal and ceramic. The software is Windows based and is not completed. The electronics have been standardized and were created in commercial grade quality using proven sub-component designs. A servo inlet for extended linear ranges was proven philosophically but flawed in dimensions and control. A highspeed GC is now seen as a possible variation from the traditional and lengthy analyses and could be relevant for Earth Sciences. In the next year, new questions will be addressed: How well do these designs actually perform in a complete and integrated system? What is the next stage of maturation? And, can we create a long-lived nonradioactive source for a portable GC/IMS-analyzer.

During the last year, various pieces of hardware, software, supporting instrumentation and protocol were assembled or created to sustain and advance our program toward the goal of this work, a sophisticated GC/IMS with a high level of automated identification of toxic or harmful vapors. The technology created in this laboratory is advanced but can be matched or exceeded by certain instrument manufacturers who are certified to make flight-hardware. Our wish is not to compete or be redundant with these other teams and our efforts are focused toward providing automated intelligence for the present and future generations of GC/IMS analyzers. The experiences of this last year have confirmed the anticipations and expectations from the last five years and shown that IMS spectra are content rich, and that spectra are founded on certain and understandable principles; in short, perceived limitations are rooted in technology not fundamentals. All this confirms and supports the decision taken years ago by NASA personnel to select GC/IMS as a flight analyzer and results bode well for the practice and application of GC/IMS on Freedom.

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 is 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.

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 philosophy 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. Applications in hazardous waste screening and industrial monitoring are envisioned for robust advanced GC/IMS technology. 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 are predicated upon the availability of qualified, affordable instrumentation in an appropriate timeframe.

Publications, Presentations, and Other Accomplishments:

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Plasma Chemical Approaches to the Development of Biofilm-Resistant Surfaces

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Students Funded Under Research: 0

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 and dishwashing and thereby present a hazard to astronauts on long-duration space missions, as in the proposed 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 first year of this three-year NASA Research Announcement (NRA) grant, which commenced formally in mid-FY95, has been concerned with plasma chemical approaches to biofilm-resistant coatings or surfaces. The work involved the collaborative efforts of a plasma chemist and a polymer chemist at ARC to produce and characterize a variety of plasma polymers, and the exposure of these and conventional polymers to pure *Pseudomonas aeruginosa* culture and their examination by a microbiologist and laboratory assistant at Southwest Texas State University (SWT).

The methodology for biofilm assessment employed previously at ARC was communicated to the SWT workers who, after numerous experiments and modifications of bioreactor techniques, were unsuccessful in replicating unpublished results at ARC that showed that a particular plasma-polymerized coating imparted remarkable biofilm resistance to several different substrata when exposed to *P. aeruginosa*. Moreover, the SWT work failed to generate the S-shaped plots obtained previously at ARC and which conformed to the general pattern of biofilm growth. On the other hand, Fourier-transform infrared (FT-IR) transmission spectra of biofilm-covered polyethylene (PE) generated at SWT were almost identical to those obtained at ARC; they provided important information regarding the presence of water, proteins and exopolysaccharides within the biofilm as well as the heterogeneity or non-uniformity of the biofilm. Thus, despite the otherwise disappointing results at SWT, a note for publication on the FT-IR spectra of biofilms produced by *P. aeruginosa* is a likely prospect.

Besides supplying numerous plasma-deposited coatings on PE film for biofilm testing at SWT, work at ARC included plasma polymerization of various fluorine-containing olefins as well as of ethylene itself. This work, involving detailed structural and surface analyses of the resulting plasma polymers, provided the required background for a later study of the effect of fluorine content on the biofilm resistance of those polymers.

For follow-on work in the second year of the NRA grant, we intend to enter into a new collaboration with a university microbiology group having special expertise in biofilm technology and research. Besides seeking a "second opinion" on the reproducibility of the prior ARC results, we propose to return to the original plan of 1) testing the notion that a particular chemical functionality (or functionalities) may impart special antibacterial properties to certain plasma polymers, and 2) of studying the biofilm resistance of plasma polymers derived from a homologous series of fluoro-olefins, as well as the biofilm resistance of a series of conventional polymers with varying fluorine content. Although there is considerable literature dealing with exposure of various commercial polymers to an assortment of microorganisms, there has been scarcely any attention given to the biofilm resistance of a family of structurally related polymers. Thus, the proposed work would help fill a gap in the open literature by leading to structure-property relationships for that important group of polymers. Also on the agenda for additional study is biofilm formation on the aforementioned fluorine-containing plasma polymers and conventional polymers when exposed to various common bacteria besides *P. aeruginosa*, such as *E. coli*, *S. aureus* and *S. epidermidis*, so as to explore the generality of the biofilm resistance suggested by the prior ARC research.

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 future space habitats (such as the proposed International Space Station) has been noted in the Abstract. 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 various artificial prosthetic devices and contact lenses. 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.

Publications, Presentations, and Other Accomplishments:

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Golub, M.A. "X-Ray photoelectron spectroscopy study of plasma-treated fluoropolymers." 6th International Symposium on Chemically Modified Surfaces, San Jose, CA, June 19-21, 1995.

Golub, M.A., T. Wydeven and L.S. Finney. Plasma copolymerization of tetrafluoroethylene and chlorotrifluoroethylene. Polymer Preprints, 36, No. 1, 107, 1995.

Golub, M.A., T. Wydeven and L.S.Finney. "Plasma Homo- and Copolymerizations of tetrafluoroethylene and chlorotrifluoroethylene." Plasmas and Polymers, (In press), 1995.

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Modeling, Monitoring and Fault Diagnosis of Spacecraft Air Contaminants

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Funding:

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Students Funded Under Research: 3

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.

1. A new three-dimensional mathematical model for contaminant release and transport has been developed. The computational technique is finite differencing using the alternating-direction implicit approach. 2. A new Implicit Kalman Filtering algorithm has been developed for contaminant detection. Unlike the traditional explicit approach, the implicit filter can be readily applied to ill-conditioned systems and allows for generalization to descriptor systems. The Implicit Kalman Filter requires significantly less computer time and storage to implement for distributed systems such as models of contaminant release and transport. 3. A new square root Implicit Kalman Filter Algorithm has been developed. This algorithm has better stability properties than the original Implicit Kalman Filter. 4. A new optimal sensor placement algorithm has been developed. It has improved properties over the suboptimal algorithm previously available in the literature.

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.

Publications, Presentations, and Other Accomplishments:

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Skliar, M. and Ramirez, W. F. "Kalman filter for discrete implicit systems." Proceedings of the 1995 American Control Conference, Seattle, Washington, June 1995, 524-528.

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Smith, G.J. "Review of a suboptimal state estimation and sensor placement algorithm for a contamination simulation." NSCORT/CSEH Tech Note #31 (internal report), Aerospace Engineering Sciences Dept., University of Colorado, 1995.

Smith, G.J. "Sensor configuration observability for a simplified contaminant transport simulation." NSCORT/CSEH Tech Note #30 (internal report), Aerospace Engineering Sciences Dept., University of Colorado, May 1995.

Capillary Electrophoretic Methods for Monitoring Spacecraft Water Quality

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Funding:

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FY 1995 Funding: $105,793

Students Funded Under Research: 0

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 and 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.

Literature survey of all relevant articles: This includes not only gathering information from scientific journals, but also gathering information from any Capillary Electrophoresis (CE) vendor published material. This has allowed us to build a sizable reference base. Evaluation of electrolyte chemistries: Over 30 variations of cation and anion electrolytes have been thoroughly tested using a Waters Quanta 4000 CE. This was done in order to determine the electrolytes which are best suited to the analysis of our target compounds. Adaptation of a Hewlett Packard bubble cell capillary for use in our CE system: The bubble cell provides a longer path length which results in an increase in sensitivity of up to 300%. Evaluation of microgravity compatible CE components: The problems associated with microgravity usage have been identified. Various bubble exclusion systems and variable volume reagent reservoirs have been tested and found to be acceptable. Preliminary breadboard microgravity-compatible CE design completion: A preliminary design for a breadboard microgravity-compatible CE has been completed and met with approval. Construction of this design is under way.

We have located and tested components which should allow us to cope with the problems of microgravity. We have shown that the CE is capable of performing the required analyses for over 80% of the target compounds and are continuing work on the remaining compounds. We have shown that it is possible to simplify the CE by using one wavelength (214 nm) for all analyses.

Questions that have arisen include:
Which type of detector will give the highest sensitivity and/or ruggedness?

What can we use as a sample injection technique to ensure good analysis precision?

How will the equipment perform in the simulated microgravity of the KC-135?

How can we further improve methods and hardware?

This year's progress represents the completion of a major milestone: The design completion for a breadboard microgravity-compatible CE. It allows us to now build and test a breadboard microgravity-compatible CE system.

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 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 (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.

Publications, Presentations, and Other Accomplishments:

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Liquid Phase Piezoelectric Immunosensors

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Funding:

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Students Funded Under Research: 3

Task Description:

Microbial contaminants in the space station can be generated by crew members and by experiments. Consequently, an automated simple monitoring and control system will contribute significantly to the success of space missions. Piezoelectric crystal sensors offer excellent sensitivity and design simplicity that make them suited for space technology. The overall objectives of the proposed research will be to develop a piezoelectric immunosensor for *E. coli*, a representative bacteria using antibodies as coatings. The technology can be adapted to monitor various pathogens and eventually several single sensors can be combined into an interdigitized array for identification of multiple agents.

The following tasks were accomplished so far; two different methods for immobilizing the antibody on the piezoelectric crystals were tested and proved to be promising. The first method involves use of protein A as a precoating of the quartz crystal followed by coating with antibody. The second technique is immobilization of the antibody on the crystal surface via glutaraldehyde cross-linking using bovine serum albumin. Both methods proved to be suitable to measure *E. coli*. A preliminary investigation was also carried out to use the coated crystals to continuously measure *E. coli* in solution, and promising results were also obtained.

In addition, three oscillator circuits to measure *E. coli* in solution were constructed as described in the literature. A forth circuit was developed and constructed in our laboratory. The performance of these circuits is being evaluated. Preliminary results obtained with the circuit developed in our laboratory were very encouraging. Successful completion of the present tasks is essential for the successful completion of other tasks of the project.

The proposed technology can be adapted to monitor various pathogens that may cause diseases and/or affect the quality of life. The 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.

Publications, Presentations, and Other Accomplishments:

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Remote Sensing for Research and Control of Malaria in Belize

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Funding:

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Joint 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 subject 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. The proposed research is aimed at improving the malaria control program in Belize. Research activities will include such diverse efforts as field and laboratory studies, using remote sensing and geographic information system technologies, mathematical modeling, developing predictions and testing new technologies, as well as training and capacity building. The hypothesis being, '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.' Studies must be conducted for each of the four known vector species in Belize. Other types of research and capacity building in RS, GIS, and mathematical modeling will be conducted to provide support for studies of malaria vector ecology. The end product will be predictive capabilities, based on remote sensing data, for each of the important malaria vectors in Belize and eventual implementation of the technology within the national malaria control program.

An Investigator's Working Group (IWG) meeting was held and research plans and timelines were developed. Timelines for establishing capabilities in Belize were rapidly superseded by bureaucratic delays in contract negotiations. Timelines for field research were fulfilled. A field survey of river associated vectors was conducted in the rainy season (in July-August 1995) with the general finding that habitats in rapidly flowing rivers, at altitudes above the coastal plain (0 to 40 m above sea level), are frequently purged by heavy rainfall. Alternatively, deep, relatively still waters of lower-altitude segments of river systems (below 40 m altitude) seem to afford some buffering of habitats so that vectors could still be detected, even during periods of heavy rainfall. It is important to note that this is a parameter that can be modeled with remote sensing data in a predictive model. However, we now know that we must more carefully define the seasonality of habitats and vector abundance in riparian zones. Presence of *Anopheles darlingi*, an important vector of malaria, was found in a highly malarious village of southern Belize. This is the first report of *An. darlingi* in southern Belize since 1946. During this reporting period work was initiated with TM data to define areas of riparian ecology for future surveys and to develop predictions for wet season distributions of *Anopheles albimanus* populations in villages in the northern coastal lowlands. Predictions will be developed and surveys will be conducted in October 1995 to test the accuracy of remote sensing-based predictions. Studies initiated by a USUHS doctoral student are providing baseline information indicating that *Anopheles vestitipennis* may be a more important vector of malaria in a variety of habitats within Belize than previously thought. To date, we do not know the environmental determinants for the presence and abundance of this potentially important species. Major work on developing the geographical information system and initiating full analysis of remote sensing data is held in abeyance by delays in getting a contract between the Henry M. Jackson Foundation and the Belizean Ministry of Health.

The objectives of this research can be met only through a greatly improved understanding of vector roles and biology, ecology, environment and disease transmission dynamics of human malaria in Belize. In other words, 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 Belizean National Malaria Control Program will have broad implications for malaria control throughout the world. This program of research has already resulted in a critical revision of our understanding of malaria epidemiology in Belize. As background, when we initiated research, predating the current NASA-funded program, 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 least four species are potentially important vectors of human malaria. We are in the process of showing that we can pigeon-hole these vectors by specific environments and seasons. Through this process, we are showing that satellite data can be used to predict where and when humans are at risk of malaria transmitted by each of the four species. Eventually, we expect to show that remote sensing-based predictive models can be used to greatly improve the cost-effective application of national malaria control measures in Belize.

NSCORT: Integrated Physiology

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Task Description:

The objective of the NASA Specialized Center of Research and Training 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. New 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, their interaction, and their regulatory mechanisms.

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 ranging from ground-based and flight experiments to service on NASA advisory groups.

Section I on cellular and molecular mechanisms examines processes that are likely to be of general importance and mediate adaptations to changing physiological demands in multiple biological systems. There are two primary areas of investigation: regulation of intracellular protein degradation in skeletal muscle, and genetic regulation of skeletal muscle hypertrophy . Section II on mineral metabolism

explores the mechanisms that are involved in bone loss and hypercalcuria as induced by immobilization or exposure to microgravity. Section III on skeletal muscle structure and function has three components studying (a) human inborn defect of oxidative metabolisms, (b) substrate regulation in skeletal muscle in disuse atrophy, and (c) changes in muscle fiber type, water content, and perfusion during unloading. 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 bed rest. Project (a) examines the role of cardiac mechanics in orthostatic intolerance, (b) the regulation of peripheral blood flow in deconditioned human subjects, and (c) baroreflex regulation of arterial blood pressure following simulated microgravity. Section V is devoted to space flight experiments (supported by separate NASA and NIH grants and contracts) and mathematical modeling of cardiovascular physiology at microgravity.

Training in these areas is provided at multiple links ranging from summer fellowships for high school students, through support of formal graduate school education to post-doctoral research fellowships. A new Ph.D. program in integrated physiology is being implemented.

This section is a brief overview of the work performed within the framework of the NSCORT. Our report includes a complete list of publications that provides more detailed and inclusive information on the full range of scientific activities within the center.

A series of studies on the structure and function of the proteasome have been completed. There is increasing evidence that the proteasome has important functions in the regulation of many cellular processes, including the regulation of growth and atrophy of tissues. A permeabilized cell systems has been developed to study the degradation of endogenous proteins, a process that may be catalyzed by the ubiquitin/proteasome system (including PA700 and PA28 that regulate the function of the proteasome). The cellular function of two oncogenes, ski and sno have been examined to determine if they play a role in muscle determination and development. The complete cDNA sequences for both have been determined in the mouse. Further studies have shown that sno transcripts appear very early during mouse development in multiple tissues suggesting that if sno is regulating muscle gene expression it must do so after muscle determination has been made.

The effects of biphosphonate (alendronate) as a countermeasure during a 3-week bed rest period have been studied. Sixteen male subjects participated in a placebo-controlled trial. A daily dose of 20 mg alendronate prevented the hypercalcuria and stone-forming propensity induced by prolonged bed rest. Further studies showed that immobilization impairs the proliferation of human osteoblasts, reducing bone formation. Bed rest may also stimulate bone resorption by removing the inhibitory action of TGFß on osteclasts.

Oxidative capacity was relatively well maintained in a patient with only 3% residual phosphorylase. Studies in 3 patients with muscle lactate dehydrogenase deficiency demonstrated preservation of pyruvate production and near normal muscle oxidative metabolism in contrast to the severe oxidative deficit in patients with complete blocks in muscle glycogenolysis. A new animal preparation has been developed for studies of muscle metabolism, isolated perfused rat hindquarter, defined infusion parameters for studies based on 13-labeled substrates and methods for measuring 20 different metabolic intermediates using enzyme-linked fluorometric assays. Different approaches to noninvasive methods to detect changes in skeletal muscle water content and fiber type during unloading continues to be examined.

Investigators from all three projects have jointly completed a major 2-week bed rest study (-6° head-down tilt) that included ten normal subjects. Major new findings include: 1. Bed rest produces an unexpected increase in overall left ventricular stiffness. However, this change is associated with a major shift to a more compliant portion of the ventricular pressure-volume relationship. 2. The cardiopulmonary reflex control of peripheral vascular resistance is augmented after bed rest. 3. Peripheral vascular responses to alpha stimulation in the leg were enhanced after bed rest.

The Space flight Experiments and Mathematical Modeling section --to which investigators from other sections have made essential contributions--includes completion of major cardiovascular flight experiments of three Spacelab flights, SLS-1 in 1991, SLS-2 and D-2 in 1993. Current work includes implementation of new flight experiments for NASA-Mir flights in 1996 and for Neurolab (Spacelab) scheduled for 1998.

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 be important on Earth.

Studies on the cellular and molecular level within the NSCORT are providing new data on fundamental mechanisms that control skeletal muscle growth and atrophy. New information on the prevention of structural and functional losses affecting the cardiovascular and musculoskeletal systems on orbit 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 bed rest. 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. Furthermore, 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.

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FY 1995 Funding: $941,000	Students Funded Under Research: 14

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.

A considerable amount of information, qualitative and quantitative, has been obtained with respect to the characteristics of initial damage to DNA, as well as to extracellular matrix. With respect to DNA damage, theoretical modeling and corresponding experimental measurements reveal that a significant amount of clustering of damage sites, both locally, over regions up to 40 base pairs and over regions extending to several kilo base pairs, occur due to a single track (lowest amount of radiation exposure) of HZE particles. As to be expected, the complexities of clustering increase as the charge of the particle increases from proton to iron. As a result of these studies, we have also discovered a new phenomenon of the production of short DNA fragments associated with multiple nearby strand breaks in a local damage clusters. These small fragments may be difficult to repair by rejoining them back to the genome and hence can lead to mutagenesis and hence to carcinogenesis. How efficiently or otherwise these fragments are rejoined back correctly to the genome, is a new question we are dealing with presently.

Recent measurements by us of double strand break induction by a variety of HZE particles have shown a decline in the yield of these types of breaks as a function of increasing LET, in contrast to an increase in Relative Biological Efficiency (RBE) for biological parameters such as cytotoxicity, mutagenesis and chromosome aberrations. This decrease in RBE is based on measurements with megabase pair-sized fragments which are supposed to be produced as a result of random distribution of breaks throughout the genome. However, as mentioned earlier, both theoretical work and experimental results recently have demonstrated non-randomness of double strand break induction in the size interval of 80 base pairs to 200 kilo base pairs as a result of clustering of breaks. When the random breaks, as well as the non-random breaks are taken into account, RBE for double strand break production becomes somewhat greater than 1.0, although still not nearly as high as the RBE for biological endpoints. In order to resolve this mystery, we have to understand how correctly the breaks in damage clusters are rejoined. In FY'95, we completed a study of overall rejoining of double strand breaks induced by a large range of densely ionizing particles. The conclusion from this extensive investigation is that the measured proportion of breaks that are not rejoined increases with LET but the proportion that are misrejoined does not change significantly.

Recently, we have developed a new human/hamster hybrid cell line ALC, which like the previously developed AL cell lines contain a sole human chromosome (#11); however, unlike the AL cells, they do not require retention of the chromosome 11p hostage locus that restrict the viability of AL cells. The paired hybrid cell lines were each irradiated with low-energy HZE particles to compare their respective sensitivities to mutation induction at the S1 locus. The S1- mutant frequency was 8-10 times greater in the ALC hybrid. The next question we are dealing with now relates to the determination of the basis for the recovery of the additional mutants in the ALC hybrid.

Previous experiments with human TK6 cells showed that cysteamine was effective in reducing the frequencies of hprt and tk-deficient mutants induced by moderately ionizing neon particles. However, such a reduction was not observed for more densely ionizing radiation iron particles. We are currently investigating whether such an absence of any reduction effect of cysteamine is due to the increased presence of clustered breaks. Experiments are also underway to test the hypothesis that cysteamine suppresses the formation of loss of heterozygosity mutation by altering the activity of topoisomerase II.

In addition to the well known phenomenon of the induction of damage to DNA, we have also found that HZE particles elicits rapid changes in the microenvironment (such as extra-cellular matrix) that are distinct from those found following sparsely ionizing radiation. In particular we have observed changes in an important mediator of epithelial integrity, the basement membrane. Furthermore, disruption of basement membrane integrity by both chemically-mediated and transgenic means promotes the

expression of mammary tumors. Following exposures to densely ionizing radiation, the basement membrane becomes irregular and discontinuous within an hour of radiation exposure. This alteration may be due to the rapid induction of proteases that degrade specific proteins of the basement membrane. Alternately, the rapidity of evidence suggests that the densely ionizing particles may cause direct physical disruption of this thin protein membrane. Further research is initiated to determine if either hypothesis is correct.

Besides radiation health research, this project has a mandate to train students and post-doctoral candidates. Two students completed their master's degree in 1995. Three students have made a considerable progress toward their Ph.D. degrees and they are expected to complete in 1996. Two post-doctoral candidates underwent training in research with HZE particles.

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, tissue 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 has emphasized the vulnerability of the lens epithelial layer for the risk of radiation-induced cataract, has 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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NSCORT: Environmental Health

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Funding:

Project Identification: 199-93-17-02 Solicitation: 94-OLMSA-04

Initial Funding Date: 1/91 Expiration: 12/95

FY 1995 Funding: $1,044,000 Students Funded Under Research: 9

Task Description:

The underlying assumption of this Center is that ground-based studies combined with past (and future) space flight data will provide data 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 research agenda of this NSCORT was executed by teams of engineers and life sciences faculty. The research progress will be presented, therefore, as team accomplishments. The Inhalation Risk Team has discovered that teflon, used as electrical insulating material in the Shuttle, was found to emit ultrafine particles when heated. Studies by the engineers in this team have determined the physical and

chemical conditions under which these particles are produced. These particles were found to be highly toxic to the lung in animal experiments. Follow-up studies conducted this year indicated that inhalation of these particles by rats dramatically increased polymorpho-nuclear leukocytes in the lung along with increased expression of messages encoding for anti-inflammatory cytokines and antioxidants. Thus, we have identified an important potential health risk for astronauts. Unfortunately, due to the abrupt cessation of funding, we will not be able to quantify the degree of risk to astronauts of this insidious problem. Risk characterization process remains incomplete.

The Human Performance Risk Team has commenced studies on the human behavioral effects of toluene which is present in the atmosphere of the Shuttle at concentrations higher than most other volatile organics degassed from plastic materials. Human performance was judged, at the outset of this NSCORT, to be a key endpoint in assessing adverse effects of toxics in spacecraft. Studies on toluene started on schedule in the third year of the grant. Use was made of the unique human exposure facility at the Medical School at Rochester. Volunteers were exposed at the occupational health limit of 100 ppm, hitherto believed to be safe atmospheric level of toluene. When the exposed volunteers were tested with complex performance tests, an adverse effect of toluene was detected. Our analyses indicated that both the composite performance and the latency scores of neuropsychological tests were significantly degraded as compared to controls. Unfortunately, due to the abrupt cessation of funding, we will not be able to continue this program, whose next step was to examine the contributions of sleep loss, infection, and other stresses added to toluene and which further threaten astronaut capabilities.

Water Recycle Team During the tenure of this NSCORT, a water recycle test system was constructed to study the problem of disinfection in recycled water systems. Iodine was used as the disinfectant. Studies this year have revealed that water-borne viruses such as the MS-2 strain of coliphage were susceptible to iodine disinfection in flask experiments but much less susceptible in the water recycle test bed. The presence of biofilm in the test bed may hamper disinfection. A new analytical method was developed to identify iodination disinfection products (IDPs). Using this new technology it was possible to identify toxic agents such as iodoform and triiodophenol in recycled water. Studies on the biological monitoring of iodine in humans were completed. In collaboration with the radiological clinic at the University of Rochester, it was possible to show in patients receiving radioactive iodine for therapeutic purposes, that the urine was the biological indicator of choice. Despite significant progress in this and previous years, important and urgent questions related to the safety of astronauts remain unanswered. The team is particularly concerned about the persistence of viruses and their resistance to disinfection in recycled water. Again, as stated for the other programs, the abrupt cessation of funding has frustrated further work on this health risk.

Quantitative Risk Team is charged with the task of translating our research findings into quantification of health risks to astronauts. This year an invited paper was presented at the 46th Annual Astronomical Congress, summarizing the achievements of this team. This paper described the risk assessment model developed and tested in the NSCORT during the last several years. Here, again this risk assessment process will be discontinued by lack of crucial data due to the untimely break in funding for this NSCORT.

The NSCORT faculty and students have developed an extensive Training and Outreach program that continues to gain momentum despite the imminent loss of funding. Several graduate students are close to completion of their thesis projects. In the outreach program, we are in the unfortunate situation of having to respond to increasing requests for presentations to schools, community groups and retirees without funding for these activities. A mobile display was constructed illustrating aspects of space environmental health that proved immensely popular with school children.

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 work place 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 work place 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 bioflims that hinder the disinfection process are directly related to these ground based public health problems.

NSCORT: NASA/NSF Joint Program in Plant Biology

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Funding:

Project Identification: 199-93-17-11

Solicitation:

Initial Funding Date: 9/94

Expiration: 8/99

FY 1995 Funding: $500,000

Students Funded Under Research: 6

Joint 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 joint 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.

There are nine research laboratories all working on related aspects of plant signal transduction. One of the goals of the research network is to enhance progress in the understanding of plant signal transduction through collaborative interaction between the laboratories bringing the combined expertise of two or more of the laboratories to bear on central problems in signal transduction. A meeting was held in Madison, Wisconsin in November of 1995 and included network PIs and administrators as well as students and postdoctoral researchers from the participating laboratories. At the 1995 meeting it was clear that impressive research progress has been made on individual network projects and that collaborative research efforts between participating laboratories are underway. There was concern expressed that no new innovative approach has yet developed that involves the coordinated efforts of a large number of laboratories in the network and represents an effort that would not be possible in the absence of the network. A meeting of network PIs is scheduled for late February 1996 to discuss design and implementation of such a project.

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.

NSCORT: BIOREGENERATIVE LIFE SUPPORT - Biomass Productivity and Sustainability of Bioregenerative Life Support Systems

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Funding:

Project Identification: 199-93-17-03
Solicitation: NRA 1990 NSCORT
Initial Funding Date: 1/91
Expiration: 12/95
FY 1995 Funding: $1,055,000
Students Funded Under Research: 65

Task Description:

The NASA Specialized Center of Research and Training (NSCORT) in Bioregenerative Life Support at Purdue University from late 1990 through 1995 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 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 NSCORT was the interactive development of crop production, food processing, and waste management 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 was utilized to determine an appropriate diet for astronauts. Overriding this research was an engineering analysis to optimize the components of CELSS and ensure that wastes are processed efficiently.

The research in this NSCORT included all of the major elements required for a functioning CELSS. A major and diverse effort focused on biomass production. The goals in this area were aimed at the efficient production of edible biomass, determination of the impact of environmental conditions on the quality and quantity of biomass production, the provision of high quality edible biomass products for food processing, and minimization of waste products. Concurrently, projects determined optimal environmental conditions for biomass production, and how to genetically engineer crops, such as rice

and cowpea, for optimal growth and nutritional value. Another project utilized cyanobacteria for production of O_2, for N_2 fixation, CO_2 assimilation, and for food. 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 major objective was to convert hydroponically grown, productive plants into acceptable, safe food products. A second objective was to specify human nutritional requirements for long-duration missions and colonization in a hypogravity environment.

Another major research area involved waste 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 biomass 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 a balanced vegetarian diet for CELSS occupants; proof of the nutritional benefits of that diet; use of cyanobacteria as a component of CELSS to help stabilize O_2 and CO_2 levels, as well as to provide combined nitrogen; and appropriate ways to stabilize the CELSS environment and processing wastes. An equal contribution of the Purdue NSCORT was the extensive training program for postdoctoral researchers, graduate students, and undergraduates, as well as the space education outreach program to K-12, civic and educational organizations, and the general public.

Continued progress was made during FY 95 preparing rice (*Oryza sativa L.*) to be a CELSS candidate species in the cereal grain category of food crops. Extensive work on photoperiod/ planting density was completed in the semi-controlled environment of the greenhouse, and intensive work was initiated on nitrogen nutrition in hydroponic culture in the highly controlled environment of the growth chamber. The semi-dwarf rice cultivar 'Ai-Nan-Tsao' was grown for 78 days at canopy densities ranging from 70 to over 1000 plants/m^2 at photoperiods of either 8, 10, or 12 hours/day. Even though floral initiation of this promising cultivar was found to be day neutral, non-edible biomass accumulates faster than edible biomass with increasing photoperiod, so a careful balance must be found between edible and nonedible biomass production. Tiller (branch) and panicle (flower stalk) number/plant declined with increasing plant density, while vegetative biomass increased. Edible yield was highest (at 1750 g grain/m^2) for 12-h photoperiods and 300 to 500 plants/m^2, whereas edible yield rate was highest (at 32 g/m^3/day) for 10-h days at the same densities. Yield-efficiency rate was 0.3 to 0.4 g grain/m3/day/g inedible biomass for all 3 photoperiods tested up to a density of 500 plants/m^2, above which it declined. A planting density of 283 plants/m^2 and a photoperiod of 10 h produced the most favorable rice yield parameters. Controlled environment work is in progress comparing the tolerance of rice to different ratios of NH_4^+:NO_3^- in hydroponic solution ranging from 0.25 to 4.0. Ammonium will be prevalent in the waste stream of CELSS, and rice is one of only a few CELSS candidate crops that tolerates NH_4^+ well. The goal of current rice research is to determine how much NH_4^+ rice will tolerate at one time and what effect it has on rice yield parameters. 'Waldmann's Green' leaf lettuce (*Lactuca sativa L.*) was used as a model CELSS leafy vegetable crop during FY 95 to develop a protocol for computer-automated control of major crop production parameters (i.e., light, CO_2, temperature) based upon feedback from real-time canopy photosynthetic rate (Pn). Initial studies compared efficiency for dynamic control of light level with that for constant high light applied to the lettuce canopy. Photosynthetic photon flux (PPF) was set daily at 80% of the level that gave maximum Pn the previous day, and was adjusted (usually upward) to find the new maximum Pn each day. Variable control of PPF gave 0.226 g dry weight edible biomass/mol of light relative to 0.187

g/mol for static control at constant 895 mmol/m^2/sec. Further studies are intended to simultaneously automate dynamic optimization of PPF, CO_2, and temperature using process-control software and the Minitron II crop canopy cuvette/controlled environment system. Another new initiative during FY 95 was to initiate intracanopy lighting studies using canopies of hydroponically cultured cowpea (*Vigna unguiculata* Walp.) growing in two controlled environment walk-in growth rooms. The planophile (horizontal-leaved) canopies of CELSS crops like cowpea, soybean, potato, and sweet potato quickly extinguish light within a closed canopy when it is provided only from directly overhead. We compare yield and productivity between traditionally overhead lighted canopies (215 watts/fluorescent lamp) and internally lighted canopies (tiered, 15 watts/fluorescent lamp). The strategy of using low power, low heating light sources within the canopy (with ballasts remotely located) should save considerable power for crop production even if the crop production rate is lower than with traditional lighting.

Work continued on a broad computer model/simulation of a CELSS. The current model, which contains over 500 flows and thousands of equations, demonstrates several aspects of CELSS atmosphere dynamics. Research also continued on circadian rhythms and heterotrophic growth in the *Cyanobacterium Cyanothece.* Research also continued on the molecular biology of nitrogenase in *Cyanothece.* The nif operon was cloned and most of this important region of the genome was sequenced. Nitrogenase rhythms are studied at the level of nitrogenase protein abundance. Control of photosynthetic activity in *Cyanothece* is being studied at the manganese-stabilizing protein (MSP) of Photosystem II. Significant advances in the isolation of oxygen-evolving thylakoid preparations allowed the extension of *in vitro* analyses. Possible modifications of MSP throughout the metabolic oscillations observed in this organism also are being investigated.

Modification of the storage protein (glutelin) gene of rice also was continued during FY 95. Because of poor expression of the modified gene in transgenic plants, a new plasmid was constructed containing a longer promoter (5 kb instead of 1 kb) of the glutelin 1 (Gt1) gene driving the modified Gt1 gene. This should result in stronger expression of the modified gene in the endosperm of rice seeds. Plant transformations were initiated with this new plasmid. A second project was initiated whose goal was to reduce the content of lignin in rice. This should enable the biodegradation of the stover remaining after seed harvest. To achieve this result an antisense of one of the genes of the lignin biosynthetic pathway was transformed into rice protoplasts and plants are in the process of being regenerated.

Research was conducted to develop a cowpea (*Vigna unguiculata* L. Walp.) transformation system using microprojectile bombardment or cocultivation with *Agrobacterium tumefaciens.* A morphogenic system utilizing embryonic axis and cotyledonary base explants has been developed to provide a target explant for transformation that can give rise to fertile plants. Besides reporter (uidA) and selectable marker genes (nptII or bar), vectors containing genes encoding either an alpha-amylase inhibitor (natural insecticidal protein) driven by a 35S CaMV promoter or a Brazil nut 2S albumin (protein with high content of sulfur-containing amino acids) driven by a phaseolin promoter were used in transformation experiments. Transformation conditions were established for optimal delivery of the genes by analysis of transient expression of a b-glucuronidase reporter gene. Organogenesis induced on medium supplemented with high concentrations (2 to 20 mM) of N6-benzylaminopurine (BA) and subsequent shoot culture under kanamycin or bialaphos selection pressure resulted in regeneration of several transgenic chimeras. Introduced genes were detected in genetically modified T0 cowpea plants by both histochemical GUS/MUG assays and PCR or Southern blot detection of transgenes. Work to obtain evidence of transferred genes in the T1 progeny (GUS/MUG assays, Biomonitor insect resistance assay, and Southern blot hybridization) is being performed.

During FY 95, food analysts studied the composition and stability of oil and protein meal from screw-press extracted CELSS candidate oilseed crops. Compositional analysis was conducted on plant parts of wheat and soybeans harvested at various stages of maturity. Data are used by the systems analysis group to model gas exchange. The composition is being studied of the nonprotein, nonnitrate nitrogen-containing compounds of controlled-environment-grown plants to assess the implications for

human nutrition. The composition is being determined for cowpea seeds harvested at various stages of maturity to determine if the levels of flatulence-causing carbohydrates and antinutrients are reduced by early harvest.

Recovery of oil from soybean, canola, and peanut was investigated. The secondary extraction of oil from seed meals obtained from a mechanical press after oil production, was investigated using aqueous methods. Preliminary investigations into the extraction of oil from whole oil seeds using enzymic hydrolysis was initiated. Investigations into the possibility of using trehalose (a carbohydrate) in food preservation also were initiated.

Optimal nutrition in a CELSS is critical for keeping the crew alive, healthy, and productive. In the most restricted nutrition scenario, a CELSS diet may be vegan (i.e. no animal products) with a limited variety of plant foods. The Nutrition Group worked collaboratively with other NSCORT investigators to define the best combination of crops to provide a balanced and appealing diet. The nutrition and systems groups are working on the design of a diet model using fuzzy logic. To date, we have helped with the design of several nutrition-input variables and the Systems group is running various scenarios using the fuzzy logic model. In collaboration with the Waste Management Group, we are defining the amount of partially digested cellulose that can be used as a carbohydrate source in diets. Collaboration with the Crop Productivity and Food Analysis Groups is creating a balanced diet that allows for maximal mineral bioavailability. Long term feeding studies investigate the ability of these diets to sustain optimal growth and mineral nutriture in rats.

Inedible plant material, generated in a CELSS, can be recycled by bioregenerative methods that utilize enzymes or microorganisms. The lignin fraction in particular represents a recalcitrant component not degraded by enzymatic methods. The white-rot fungus *Pleurotus ostreatus* effectively degrades lignin and produces edible mushrooms. An unstructured model has been developed for the growth of *P. ostreatus* in a solid-state fermentation system using lignocellulosic plant materials from *Brassica napus* (rapeseed) as a substrate at three different particle sizes. A logistic function model based on area was found to fit the surface growth of the mycelium on the solid substrate with respect to time, while a model based on diameter alone did not fit the data as well. The difference between the two measures of growth was particularly evident for mycelial growth in a bioreactor designed to facilitate a slow flow rate of air through the 1.5-cm-thick mat of lignocellulosic biomass particles. The result is consistent with the concept of competition by the mycelium for the substrate (i.e., a two dimensional effect) which surrounds it, rather than just substrate, which is immediately available to single cells (a one dimensional effect). This approach provides a quantitative measure of *P. ostreatus* growth on lignocellulosic biomass in a solid-state fermentation system. The model is fitted to experimental data which show that the best growth is obtained for the largest particles (1 cm) of lignocellulosic substrate. The application of this model, together with an understanding of the metabolism of *Pleurotus*, led to the development of a novel bioreactor which facilitated perfusion of humidified, oxygen enriched, air through the plant biomass. Complete disappearance of the plant material was achieved after the 60-day fermentation period, upon the onset of mushroom growth. This type of biological subsystem has exciting potential to treat inedible plant materials on a stand-alone basis.

The Systems Analysis group continued work on diet selection, plant growth, and gas exchange. Work in the area of diet selection led to the addition of a fuzzy expert system to the diet optimizer, which provides a nutritionally justifiable quantification of the relative acceptability of candidate diets. In addition, the relative importance of nutrients and their potential toxicity can now be taken into account in the optimization. Finally, the expert system/optimizer program has been interfaced with a user-friendly database so that varied nutritional data can be accessed and used quickly. The database includes USDA nutritional data as well as new data from the NSCORT. The database interface has proven to be a useful tool by providing easy access to USDA data, and has been placed on the World Wide Web for the benefit of the nutrition community at large. To realize acceptable diets in the context of closed environments such as CELSS, a methodology for the control and optimization of plant growth under global system constraints will be necessary. We have begun simulations, based

on Biomass Production Group data, to develop estimation and growth optimization algorithms. These algorithms will be implemented on the new real-time data collection and control environment developed for the Minitron II plant-growth system. The new software augments the capacity of existing facilities to control the growth chamber environment. Systems Analysis has collaborated extensively with Crop Productivity, Food Analysis, and Nutrition groups in developing a gas-exchange model for CELSS. Time series data on plant composition have been collected using destructive sampling. We have used these data to calculate changes in plant assimilatory quotient (AQ) over time. The nature of the data permits us to calculate the contribution of specific plant parts to this ratio. This information has been used to develop a global CELSS gas-exchange model based on logistic equations. The model will permit investigation of the dynamic relationship of diet, plant growth, and gas exchange. Simulations have yet to be completed.

The NSCORT Education and Outreach Office was established February 1, 1994. The position of Education Coordinator was created to define, organize, and implement an education and outreach program. Responsibilities included development of educational and presentation materials that would link NSCORT research with curriculum concepts and Earth benefits as well as conducting presentations and workshops. A summary of Education and Outreach products and activities includes production of a 15-minute media overview of NSCORT research and its Earth Benefits, a kit of samples representative of each NSCORT lab, a poster and free-standing visual display depicting six general areas of research, and design and production of an NSCORT brochure and annual report.

Activities included a total of 94 presentations and participation in 3 poster sessions and 5 state and national conferences. Presentations were delivered on 14 different space life sciences topics and involved 18 different NSCORT members. Establishment of a World Wide Web site was completed and 15,000 documents were downloaded during the first 6 months of operation. Media outreach extended to television and radio interviews, provision of information for general print articles, and response to numerous phone and electronic mail requests. The NSCORT Education and Outreach Office completed cooperative activities with the Purdue School of Aeronautics and Astronautics (co-hosting a seminar speaker and design of the Indiana Space Grant Consortium Summer Teachers' Workshop). Cooperative efforts included inclusion in presentations of CELSS-related research at Johnson Space Center and Kennedy Space Center. Frequent contact was maintained with individuals at both field centers as well as NASA Space Life Sciences Headquarters.

The protocols, technologies, and information generated from higher plant biomass productivity research by NSCORT has direct application to the fledgling Controlled-Environment Agriculture (CEA) industry as well as to the CELSS program. There has not previously been a consistent funding source for research and development in CEA due to lack of profitability in most sectors of the industry, and the venture capital that has been available has been applied directly to greenhouse crop production. As optimizing environments and phasic growth requirements of CELSS crops become better defined by NSCORT research, their implementation for specific candidate species will be better visualized as automated and/or robotic crop production systems. Development of such technologies, as well as the scientific data and information generated from crop production research using modified controlled environments, will be just as valuable for CEA on Earth as for life support in space. No breakthroughs in plant-growth-lighting technology or development of a clean, efficient energy source are anticipated soon. The best hope for near term profitability in the CEA industry will be the development of computerized control algorithms to simultaneously optimize temperature, lighting, and CO_2 regimes at different stages of crop development. Creation of active control systems for CELSS candidate species in the NSCORT program will have immediate application for commercial CEA crop production on Earth.

Work with cyanobacteria will provide a better understanding of photosynthetic productivity under different environmental growth conditions. Analysis of the periodicity in N_2 fixation and O_2 evolution will provide a model system for the analysis of circadian rhythms in other organisms, including humans. Preliminary work on *Cyanothece* as a component of CELSS led to a successful

USDA grant application that will allow study of these mechanisms in more detail. A goal is to study the molecular and physiological basis of the regulation of photosynthesis and N_2 fixation in a single cell. Such studies clearly are of importance to agriculture and may be valuable for crop productivity. Long-range experiments will help us understand how N_2 fixation in cyanobacteria can be used for fertilization of crops, such as rice. Furthermore, N_2-fixing cyanobacteria have the potential to provide an inexpensive source of protein in agriculturally impoverished regions. *Cyanothece* requires little input of materials, can be grown in simple facilities with few personnel requirements and can provide a balanced protein supplement in large quantities. Additional Earth benefits may reside in unique natural products. *Cyanothece* produces an extracellular matrix that appears to be a hydrocolloid, and may aid food-processing applications.

The Earth has about 5.6 billion people today and 40% of them (2,240,000,000) eat rice as their primary source of calories. All demographic projections indicate these numbers will more than double in the next 40 years. Today, about ten percent of the population are thought to be malnourished. Part of this malnutrition is due to the fact that rice seed protein is deficient in lysine and tryptophan. These essential amino acids have to be obtained through diet--the body cannot make them. Thus, an increase in the levels of lysine and tryptophan in protein of the rice grain would have a huge influence on the nutrition of people on Earth.

Cowpea (*Vigna unguiculata L.* Walp.) known also as southern pea or blackeye pea, is one of the world's most important legume food crops. Annual production is estimated to exceed 2.5 million metric tons of dry beans harvested from over 9 million hectares. Cowpea is widely grown by subsistence farmers in Central and West Africa as a significant dietary protein source. In the USA, India, Australia and several countries of Southeast Asia and Central and South America, cowpea also has important horticultural, agronomic, and processed food (e.g. protein concentrate, canned) use. Cowpea is a valued source of dietary protein in tropical areas of the world, particularly where drought is prevalent. However, cowpea protein is deficient in sulfur-containing amino acids and must be combined with cereal protein for balanced human nutrition. Deficiency in sulfur-containing amino acids could be alleviated by transforming into plants and expressing in leaves and seeds genes that encode proteins with high contents of methionine and cysteine, e.g. Bex, zein, or nodulin-21. These transgenic plants would substantially alter the protein nutrition quality for people who are dependent on cowpea as the primary source of dietary protein.

Benefits to Earth from crop and food compositional research include knowledge gained concerning differences in nutrient composition of field-grown versus hydroponically-grown plants, and how to manipulate nutrient solutions and other conditions of a hydroponic system to influence the nutrient composition of plants. Information gathered to determine the edibility of various plant parts, and any concerns with a limited vegetarian diet can have applications on Earth. Research on toxic/antinutritive factors and ways to minimize the levels or overcome their effects by processing has implications for foods eaten on Earth. Technological development in utilization of non-traditional food sources (e.g. cowpea) will result in nutritionally-improved novel products, which is especially significant to some third-world countries and to those seeking a vegetarian diet. The optimization study of a CELSS diet indicated the importance of variety in the diet as well as the necessity of providing vitamin and mineral supplements. This will aid in composing nutritionally-balanced vegetarian diets both on Earth and in space. All studies related to developing space-compatible food processes, such as freeze-drying, may lead to improved food quality and development of new products as well as automation of some small-scale processes. Multi-functionality and durability will be built into some small-scale equipment or utensils by consolidation of similar unit operations. These developments may result in a revolution of the modern kitchen equipment and should benefit institutional food service, caterers, and consumers on Earth.

Nutrition studies on CELSS diets have world-wide applications. Diets of many developing countries consist solely of limited plant foods. Furthermore, there has been an increase in the percent of total calories from plant foods during the past several decades in the U.S. Therefore, nutrition studies

concerning vegetarian diets can be applied to Earth-related issues. In addition, providing information concerning the nutritional adequacy of plant material not typically consumed by humans (stems, leaves, roots) may be of benefit to agriculturally disadvantaged countries in that the potential use of these plant parts could maximize edible plant biomass in these developing countries.

The integration of waste processing into a CELSS involving both physical/chemical and bioregenerative technologies must consider parameters of safety, efficiency, reliability, robustness, and environmental compatibility in the context of space travel. In addition to presenting numerous challenges to the research, these factors have driven development of research strategies and choices of reagents and conditions for processing inedible plant materials. A benefit of the project's selection pressures has been spinoff of CELSS technology for terrestrial applications. Fundamental studies on the nutritional requirements, growth kinetics, and control of *Pleurotus ostreatus* grown on lignocellulosic solids have led to a novel bioreactor design. This development will contribute to processes for biological delignification of wood. Bioprocesses are attractive since they could help to moderate the negative environmental impacts of chemical pulping methods currently in use. The potential economic and environmental impacts are enormous given the $122 billion US market value of pulp and paper. The control, modeling, and bioreactor development for production of fungi also could offer improved methods of mushroom production for human consumption. A fundamental understanding of fungal nutritional requirements will help develop their use for treating poor quality forages. Growth of *P. ostreatus* could improve the feed value of such forages by providing a means of *in situ* delignification prior to animal feeding. The dry weight composition of mushrooms is 10 to 27% protein, 46 to 80% carbohydrate, 1 to 5 mg/g thiamine, and 40 to 50 mg/g niacin; mushrooms offer additional useful nutrients. Fundamental insights into structure/function relationships between cellulose, pretreating agents, and enzymes have resulted in new methodologies for large scale recycle of cellulosic (municipal) wastes, agricultural residues, and renewable resources into value-added products using environmentally compatible chemicals and biological agents. In addition, new chemistries and biochemical agents for the preparation of materials with potential use in purifying biopharmaceuticals and food products have resulted. Several patent applications are filed, and further industrial input is being sought for these technologies.

The development of strategies for controlling plant growth in controlled environment agriculture will provide agriculturists on earth greater determinism in the end product of plant growth. Considerable time lag between environmental input and crop harvest limits the effectiveness of current crop-planning strategies, if such strategies are in use at all. The research directions of the NSCORT Systems Group include the monitoring of plant functions via state estimation, optimization techniques to maximize plant growth at a minimum cost, and dynamic crop scheduling/planning. These techniques can be applied together to maximize yield, timing of harvests, and to optimize the use of resources, virtually eliminating the time-lag problem. This would allow growers to better respond to market and consumer pressures by giving them the ability to customize or individualize their crop product.

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NSCORT: Vestibular Research/(NIH)

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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-collic) reflex and three 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 1 and 2 will bring on line new devices designed specifically to study otolith systems. Modelers involved in Projects 1, 2, 4 and 5 will 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. Recordings proposed in Project 2 will yield the first three-dimensional analysis of otolith signals at the level of vestibulo-spinal neurons. 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 1 and 4 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 1 and 5 employ non-linear systems models to simulate how central nervous system control of head or body position interacts with body biomechanics. Project 2 uses new neural network modeling approaches to analyze the function of circuits that incorporate the known connectivity of vestibulo-collic pathways. 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 will also have 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: Experiments supported by the angular and linear vestibulocollic reflexes in humans project have characterized the dynamic properties of vestibulocollic reflex stabilization of the head in humans during yaw and pitch rotations about axes passing through the head. When subjects attend to the task of stabilizing the head, excellent stability is observed in both planes at low and high frequencies while interference between head motions induced by neural and mechanical forces degrades stability at intermediate frequencies. When subjects are distracted, low frequency stability is lost in yaw and reduced in pitch. The difference is likely due to the contribution of otolith organs to the pitch response. Larger errors are also observed at mid frequencies. We have now prepared a closed loop dynamic model of reflex and mechanical head stabilization which accounts for these dynamic properties in the yaw plane. The model is being extended to the pitch plane. We are also preparing a biomechanically accurate model of the head and neck system, which now includes 18 pairs of muscles acting about 8 joints. Accurate estimates of attachment sites of these muscles derived from data in the literature and the landmarks of the computer representations of the bones have allowed us to compute the moment arms of each muscle as a function of the head-neck posture. Interestingly, muscles typically have much larger moment arms in pitch than in yaw, which may explain our observation that the neck is stiffer in pitch than in yaw.

PROJECT 2: LINEAR VESTIBULOCOLLIC REFLEX IN PRIMATES: We continue to study electromyography responses produced by reflex excitation of neck muscles in squirrel monkeys and cats during externally applied linear and rotational motions. Our most general research finding during this fiscal year has been the greater magnitude and consistency of neck reflex responses to rotational tilts in comparison to linear accelerations, even when similar 'g' forces are involved in the two types of stimulation. Thus, we are now concentrating on vestibulo-collic responses to rotational tilt. Two preliminary findings have emerged in the squirrel monkey: (1) The relative importance of side-to side roll motion in neck reflex excitation is greater in the monkey than we observed in previous and ongoing cat studies, where pitching motions elicited larger responses. This suggests that the exact morphology of the head and neck mechanical system, which varies across species, may determine the spatial organization of neck reflex responses. (2) Vestibulo-collic reflex excitation undergoes spatial reorganization when the body is reoriented with respect to gravity. Our working hypothesis is that a compensatory vestibulocollic 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. The implication of this preliminary finding is that differing neck reflex neuronal circuitry is called into play depending on context, with the overall system being more complex than previously appreciated.

PROJECT 3: HEAD STABILIZATION IN THE MONKEY: Significant progress has been made during the last year in the Goldberg-McCrea-Boyle project. Although there have been no publications to date, some of our data were presented at the annual meeting of the Center in Portland last November. We are currently analyzing our data, and are preparing several reports for journal submission. The main findings that are to be published relate to 1) the gain changes in the vestibulocollic reflex linked to active gaze pursuit and 2) the absence of voluntary head movement signals on secondary vestibulo-spinal neurons. Dr. Greg Gdowski joined the project at last in the summer of 1995, and has had an immediate impact. Dr. Gdowski has developed new analytic software for quantitative analysis of active and passive head movements and single unit activity, and has played an active role in developing a new experimental setup for recording single unit activity in head free squirrel monkeys trained to generate accurate head following movements to targets. He will be presenting some of his preliminary findings at the Neural Control of meeting this spring.

PROJECT 4: ORGANIZATION OF POSTURAL CONTROL: VESTIBULAR PROCESSING: What is the contribution of biomechanical and vestibular constraints to postural control strategies? We have identified several biomechanical constraints on postural coordination: 1) Joint torques, calculated with forward dynamic simulation show that the "hip strategies" involves active hip torque, not just kinematic trunk flexion; 2) Pilot studies show that our dynamic optimal control model correctly predicts changes in strategy selection with changes in initial posture; and 3) Two diagonal force and EMG patterns characterize postural responses in multiple directions simplifying neural control of postural equilibrium. We have also investigated the following vestibular contributions to postural coordination: 1) An otolith signal could trigger a hip strategy since subjects begin to use a hip strategy at a particular threshold of vertical head acceleration; 2) We have found that patients with profound, bilateral vestibular loss with conventional clinical testing have varying degrees of preservation of otolith control of eye movements (off axis). We will verify this finding with linear VOR and correlate postural coordination measures; 3) Our computational model uses coherence functions to accurately characterize postural coordination under altered sensory condition but suggests an independent mechanism for controlling slow drift of posture under 1 Hz; 4) Patients with profound, bilateral loss of vestibular function can orient their trunks and legs but not their heads with respect to altered gravito-inertial vector in a rotating room suggesting that somatosensory control may be sufficient for postural orientation; and 5) We discovered that, unlike profound loss of vestibular function, profound loss of somatosensory function delays and/or eliminates equilibrium responses.

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. Firstly, a linear system analysis of stance is underway, using sinusoidal translations of the support surface at different horizontal orientations. Data from intact cats have been collected and analysis is proceeding. A second study is underway to examine head stabilization during stance. Preliminary results indicate that the dorsal neck muscles are not recruited during translations of the support surface, suggesting that the head is stabilized only passively, by inertia. Nevertheless, static head position (left, right, up, down, centered) has a significant effect on recruitment of limb and trunk muscles during translation. Data have also been collected to examine the postural responses that may accompany voluntary head movements during stance.

Projects 1 and 4, 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 1 is helping us to understand the problems experienced by such patients and is suggesting ways in which they could be ameliorated.

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 4 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, may provide the necessary control. Tasks which require dynamic head and trunk orientation, however, may require accurate vestibular. 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. 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 4 of Project 1 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. Our model therefore has the potential for 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 1 and 4 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 2, 3 and 5, which examine vestibulo-spinal systems at levels of detail that are not possible in humans. Projects 2 and 5 are revealing the detailed patterns of reflex muscle activation that underlie postural stabilization. Project 3 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.

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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 scientific goals and objectives of this NSCORT have been to gain fundamental insight into the role of gravity in cellular and developmental biology by a broad-based, but focused, approach, using both a breadth and diversity of cellular and developmental systems, linked by a common unifying hypothesis. This approach had the advantages of utilizing both plant and animal systems, of organizational diversity, from subcellular to cellular to tissue to whole organism, and ranging over developmental time. Thus, commonalities between systems could be identified, sensitive and insensitive systems could be discriminated, and windows of gravitational sensitivity could be recognized. The unifying hypothesis presented a beginning to the consideration of the basis of gravitational sensitivity that would explain such sensitivity in living cells. Substantial amounts of work have been fundamental, basic characterization and experimental understanding of cellular and developmental systems, essential for knowledge advancement, in general, and crucial to designing and conducting manipulated gravity studies. In addition, specific progress and information has been obtained, directly relative to the role of gravity. Some examples of these kinds of achievements are identified, in summary fashion, here. Briefly, Center research has shown significant impact of altered gravity environments on cells of the immune system (macrophages and lymphocytes), and identified protein kinase C as a candidate gravity sensitive component of the signal transduction cascade. Gravitational sensitivity has also been demonstrated in EGF-receptor transfected murine fibroblasts, establishing an improved model system. Further, it is now clear that some organs/tissues have accelerated development in microgravity (e.g., embryonic pancreas), while others have retarded development (e.g., mineralizing metatarsals). Developing heart exhibits a window of stage-specific retardation of differentiation, and brine shrimp development is accelerated in the microgravity environment of space flight. Center research has also raised questions about the reliability of clinorotation as a model for microgravity, as the result of direct comparisons of plant cells in clinorotation and microgravity environments. A study of the control of cell division by a cell-surface-active negative regulator of mitotic progression showed no effect of microgravity, documenting that some signal transduction events are refractory to that environment. It has been shown that trace amounts of heavy metal ions (Ag+), a possible contaminant of closed space craft environments, alters cytoskeletal function in cell shape control, raising questions about combined effects of microgravity and other agents. Center research has also shown gravity effects on two different self-assembly systems, lipid assembly into liposome membranes and polyoma coat protein assembly into capsomeres and capsids. Additionally, a marine model developing cornea system has been described, and differences between Spacelab and control avian corneas have been identified. Our newest Center Project has already demonstrated dramatic differences in the expression of SAUR genes in transformed *Arabidopsis* in a microgravity environment.

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 are listed here: 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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Mechanical and Molecular Stimuli for Normalizing Muscle Mass During Unloading

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Task Description:

Unloading of postural skeletal muscle, as seen during space flight or ground based models such as bed rest 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) hind limbs 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-1) in the mediation of muscle mass conservation, the levels of IGF-1 protein, mRNA and receptors will be measured. Later phase experiments will directly manipulate IGF-1 levels in a single target muscle during chronic unloading with or without training.

The following methods and techniques have been developed: a Ribonuclease protection assay for determination of Insulin Like Growth Factor-1 (IGF-1) and messenger ribonucleic acid (mRNA) levels; methodology for the extraction of IGF-1 protein from muscle tissue; and a radioimmunoassay procedure for the detection of muscle IGF-1 protein.

A pilot study designed to elucidate the potential role of IGF-1 functioning as an autocrine growth factor mediating compensatory muscle hypertrophy has been completed. This study will provide foundational data for the remainder of the grant objectives. A manuscript resulting from this study is in preparation and an abstract reporting preliminary findings has been accepted by the American College of Sports Medicine for presentation at the 1996 annual meeting.

Briefly, the findings of this study indicate that skeletal muscle IGF-1 mRNA and protein are upregulated within three days of the imposition of an overloading stimulus on skeletal muscle. The observed increase in muscle IGF-1 protein is not dependent on circulating growth hormone. The time course of increases in skeletal muscle IGF-1 protein production precedes that of the increases in muscle protein content indicative of muscle hypertrophy. The timing of increases in IGF-1 mRNA production

indicate that the observed increases in IGF-1 protein are in response to pre-translational events, most probably increased transcription of the IGF-1 gene.

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-1) has been shown, in cell culture models, to increase muscle cell protein production. IGF-1 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-1 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.

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Transduction Mechanisms in Vestibular Otolith Hair Cells

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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.

The results of earlier *in vivo* studies suggested that hair cell regeneration in the vestibular otolith organs might involve the mitotic production of new hair cells. To examine the role of mitotic division in hair cell recovery, we injected bullfrogs with 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue incorporated into mitotic cells. Using BrdU immunocytochemistry, we then measured the number, macular location, and cell morphology of BrdU-labeled cells at varying survival times.

Cell proliferation was seen in normal and gentamicin-treated animals. BrdU-labeling in the sacculus was more extensive than in the utriculus, consistent with the greater damage caused by gentamicin to

the former organ. BrdU-labeled cells were initially seen in the macular margins and, within the maculae, immediately adjacent to the basement membrane. The latter cells had spherical cell bodies and, unlike typical supporting cells, did not project to the lumenal surface. At later survival times, BrdU-labeled cells were located further from the basement membrane and displayed mitotic figures, suggesting that progenitor cells underwent cell division at more apical positions.

The total number of BrdU-labeled cells in normal and gentamicin-treated animals increased with survival time. Cell proliferation, however, was insufficient to explain the amount of hair cell regeneration seen in the vestibular otolith organs. At early survival times, large numbers of hair cells with immature hair bundles were seen in gentamicin-treated animals before the arrival of progenitor progeny. Moreover, the total number of proliferating progeny at late survival times was still significantly lower than the number of regenerating hair cells. The great majority of regenerating hair cells in the vestibular otolith organs were not BrdU-labeled, suggesting that hair cell recovery in these organs was primarily determined by non-mitotic mechanisms. These mechanisms might include hair cell migration from undamaged macular regions, hair bundle repair in damaged hair cells, or the conversion of undamaged supporting cells into hair cells (see below).

Rhodamine-conjugated phalloidin was used to label filamentous actin in normal and gentamicin-treated organs. This technique clearly differentiated hair cells and supporting cells and improved the visibility of immature hair bundles, allowing us to more closely examine the processes of hair cell degeneration, scar formation, and hair cell regeneration. In normal animals, phalloidin strongly labeled filamentous actin in intercellular adherens junctions at the apical surfaces of marginal cells and, within the sensory epithelium, the apical surfaces of hair cells and supporting cells. It also strongly labeled the sensory hair bundles and the cuticular plates of hair cells. By contrast, little phalloidin labeling was observed within supporting cells.

In normal animals, phalloidin labeling above the lumenal surface was abolished 2 days after gentamicin treatment, confirming that this treatment resulted in a profound loss of hair bundles throughout the central sacculus and within the utricular striola. Phalloidin labeling of hair bundles was largely restored in both the saccular and utricular striola at later survival times, consistent with the appearance of regenerating hair cells. The distribution of phalloidin labeling in intercellular adherens junctions, except in regions of extensive hair cell damage, was continuous throughout the sensory macula. Small epithelial holes, presumably left by individual extruded hair cells, were observed throughout the saccular macula. Scar formations, composed of the expanded processes of neighboring supporting cells, were seen surrounding the degenerated remnants of hair cells or the epithelial holes left by extruded hair cells.

Scar formations in the bullfrog vestibular otolith organs, unlike those in mammalian vestibular organs, were rapidly replaced by regenerating hair cells with immature hair bundles. By seven days postinjection, immature hair bundles were seen throughout the saccular macula, although scar formations were still visibly apparent and hair bundle density was less than in normal animals. Hair cells with immature hair bundles in gentamicin-treated animals were surrounded by fewer supporting cells than hair cells with more mature hair bundles. Mature saccular hair cells, for example, typically had five neighboring supporting cells, although they could be surrounded by from four to seven of these cells. Immature hair cells, by contrast, typically had four neighboring supporting cells. The number of supporting cells surrounding epithelial holes or taking part in scar formations in gentamicin-treated animals was similar to the number of supporting cells surrounding mature hair cells in normal animals, indicating that this decrease in supporting cells occurred after scar formation. Supporting cells with hair cell-like characteristics, i.e., cuticular plates and immature hair bundles, were often seen in scar formations. We also observed a significant decrease in the overall number of supporting cells after gentamicin-treatment, suggesting that hair cell recovery in damaged macular regions was primarily determined by the conversion (transdifferentiation) of undamaged supporting cells into hair cells. We hypothesize that the loss of intercellular contact between hair cells and supporting

cells upon local hair cell death triggers intracellular signals in supporting cells which initiate this conversion (transdifferentiation) process.

Regenerating hair cells in earlier *in vivo* studies were seldom BrdU-labeled, suggesting that hair cell regeneration was largely due to non-mitotic mechanisms. We considered the possibility that BrdU, because it was not administered continuously, was not equally available to all proliferating cells. To rule out this possibility, we repeated our earlier studies of cell proliferation and hair cell regeneration in saccular and utricular organ cultures.

Excised saccular and utricular maculae were incubated in Wolfe-Quimby incubation media (GIBCO) and placed, hair bundles upward, in sealed culture chambers. Cultured organs were maintained for 7-14 days, replacing half of the culture medium with fresh culture medium every two days. We assessed the morphological integrity of normal cultures using Nomarski optics and their vitality with vital dye exclusion. Saccular and utricular cultures were maintained with little cell damage for up to 7 and 11 days, respectively. Within this time frame, the otolith membranes of cultured organs were only marginally restored, allowing good visibility of hair cells and their sensory hair bundles. In cross-section, the cell and hair bundle morphology of cultured organs was similar to that of normal animals. Saccular and utricular maculae cultured with intact otolith membranes and nervous innervation maintained normal cell and hair bundle morphology for longer periods than maculae cultured without these structures.

Organ cultures pre-incubated for 6 hrs in culture medium supplemented with 250 μM gentamicin sulfate displayed extensive cell and hair bundle damage by 2-3 days. This damage, as *in vivo*, was seen throughout the saccular macula but was restricted in the utricular macula to the striolar region. Cell proliferation in both normal and gentamicin-treated cultures consisted of a small number of condensed BrdU-labeled progenitor cells and a large number of diffuse BrdU-labeled progeny. BrdU-labeled cells in normal saccular cultures were seen in the macular margins and throughout the sensory macula. In the utriculus, BrdU labeling, although seen throughout the medial extrastriola, was concentrated on the medial striolar border. Cell proliferation, especially in the saccular and utricular margins, was higher than in vivo and was up-regulated in gentamicin-treated cultures. The majority of BrdU-labeled progeny, as in vivo, were supporting cells, although BrdU-labeled hair cells were seen in both normal and gentamicin-treated cultures.

Many hair cells with immature hair bundles were also seen in gentamicin-treated cultures, indicating that hair cell regeneration was supported by our culture conditions. The distribution of stereociliary height in gentamicin-treated cultures was also shifted to lower values than in normal cultures, reflecting the loss of existing hair cells and the creation of new hair cells with immature hair bundles. This distribution was also broader than that in normal cultures, suggesting that immature hair cells were forming and maturing throughout the incubation period. *In vitro* hair cell recovery was slower and less complete than *in vivo*, beginning within 2 days of gentamicin treatment and restoring hair bundle density to 50-75% of its normal value by 7-9 days.

To determine if hair cell regeneration could take place in the absence of cell proliferation, we cultured normal and gentamicin-treated organs for 7-9 days in culture medium supplemented with 25 um aphidicolin, a blocker of nuclear DNA replication. Aphidicolin was highly successful in blocking cell proliferation, eliminating diffuse BrdU-labeling in both normal and gentamicin-treated organs. Condensed BrdU-labeled cells, on the other hand, were few in number in organs cultured both with or without aphidicolin. Normal organs cultured in aphidicolin-supplemented medium had normal cell and hair bundle morphology. In gentamicin-treated organs, hair cell recovery was similar in organs cultured in normal or aphidicolin-supplemented culture medium, confirming that cell proliferation was not required for hair cell regeneration and suggesting that early hair cell recovery was largely due to non-mitotic mechanisms.

We used pan-cytokeratin antibodies, a putative supporting cell marker, to examine the distribution of cytokeratins in the vestibular otolith organs. These antibodies, as in mammals, did not immunolabel hair cells. They did, however, strongly immunolabel the apical surfaces and, to a lesser extent, the cytoplasm of supporting cells, proving that pan-cytokeratin antibodies are useful cell-specific markers of supporting cells.

Gentamicin treatment induced a small number of supporting cells to lose their cytokeratin immunolabeling. Supporting cells with weak cytokeratin immunolabeling, unlike typical supporting cells, had round lumenal surfaces and did not contact other weakly labeled supporting cells. Hair cells with immature hair bundles, unlike those with mature hair bundles, also demonstrated weak cytokeratin immunolabeling, providing further evidence that supporting cells can convert into hair cells following local hair cell damage. We are now analyzing cytokeratin labeling in gentamicin-treated organs in more detail to determine when, where, and how changes in cytokeratin expression take place.

We have also used immunocytochemical techniques to identify and determine the intracellular distribution of several Ca^{2+}- binding proteins in normal and gentamicin-treated vestibular otolith organs. In normal organs, immunolabeling was not seen in supporting cells. Immunolabeling in hair cells was consistent with the presence of calbindin (CaB), calmodulin (CaM), calretinin (CaR), and parvalbumin (PA). S-100, previously shown to label striolar hair cells in fish vestibular otolith organs, did not label hair cells in the bullfrog. CaB and CaM immunolabeling was also seen in myelinated afferent axons and unmyelinated afferent nerve terminals, particularly in the central sacculus and utricular striola.

Within the sensory macula, the labeling patterns of some Ca^{2+}- binding proteins were localized to specific macular regions. In the utriculus, for example, CaM and PA immunolabeling was stronger in striolar than in extrastriolar hair cells. Ca^{2+}- binding protein immunolabeling, with the exception of CaR, was found in both the cell body and the hair bundles of vestibular hair cells. The latter immunolabeling was restricted to the apical tips of the stereociliary array, suggesting a functional role for these proteins in mechanoelectric transduction and adaptation. We are testing, in separate studies, the hypothesis that differences in the dynamics of calcium buffering can control the rate or extent of adaptation in different hair cell phenotypes.

Hair cells on the saccular margin were more darkly labeled for many Ca^{2+}- binding proteins than hair cells in the central sacculus. This labeling pattern suggests that hair cells in the macular margin, known to be a growth zone, up-regulate Ca^{2+}- binding protein expression during development. We now are studying the distribution of Ca^{2+}- binding immunolabeling in gentamicin-treated organs incubated in aphidicolin-supplemented culture medium to test the hypothesis that gentamicin treatment induces dynamic changes in Ca^{2+}- binding protein expression in hair cells and supporting cells. Interestingly, CaB and PA immunolabeling, although not up-regulated in typical supporting cells, are elevated in immature hair cells and in supporting cells with hair cell-like characteristics after damage to existing hair cells. We have also begun to study the distribution of these proteins at the electron microscopic level, correlating immunocytochemical labeling in hair cells and supporting cells with their cell and hair bundle morphology.

We have, in collaboration with Dr. Peter Gillespie of Johns Hopkins University, immunocytochemically demonstrated that a myosin Ib isozmye, thought to underlie adaptation in saccular hair cells, is also found in the cell bodies and hair bundles of utricular hair cells. Myosin immunolabeling in the hair bundles of saccular and utricular hair cells is specific and is blocked when myosin Ib antiserum is pre-adsorbed with a peptide sequence from bovine myosin Ib. In the utriculus, hair bundle immunolabeling is seen in both striolar and extrastriolar hair cells, indicating that both adapting and nonadapting hair cells have myosin Ib in their hair bundles. Interestingly, there is also a significant difference in the amount of myosin immunolabeling in the hair bundles of hair cells in the inner and outer striolar rows. Since hair cell in these rows are, respectively, slowly and rapidly adapting, this result suggests that subtle differences in the number of myosin molecules in the hair

bundle may be at least partially responsible for differences in the adaptation kinetics of utricular hair cells. Myosin hair bundle immunolabeling is also qualitatively stronger in developing hair cells located on the macular margins of the saccular and utricular maculae. We are now attempting to determine if myosin hair bundle immunolabeling is up-regulated in hair cells and supporting cells during hair cell regeneration.

Progress on our patch clamp studies has been slow while our laboratory space has been expanded and renovated for whole-cell patch clamp studies. We have, however, completed our *in vivo* and *in vitro* studies of hair cell regeneration in the bullfrog vestibular otolith organs following aminoglycoside ototoxicity. These morphological and immunocytochemical studies have demonstrated that supporting cells as well as hair cells are important for hair cell regeneration. This observation has important implications for our biophysical studies and has enabled us to recognize regenerating hair cells at varying developmental times. We have also completed two patch-clamp setups, optimized our procedures for obtaining dissociated hair cells and macular slices from normal and regenerating organ cultures, and successfully obtained gigaohm seals from both saccular and utricular striolar hair cells.

The progress of our physiological studies has been recently facilitated by the recent arrival of a graduate student (Mr. Carl Nelson) who is studying the kinetic properties and time of appearance of individual voltage-dependent conductances in normal and regenerating hair cells. These studies will initially focus on the sacculus because the properties of its hair cells are well known from previous studies and on the utricular striola because its hair cells are larger and easier to record than those in extrastriolar regions. In these experiments, we will contrast the passive and active membrane properties of supporting cells and hair cells in normal organs. We will then examine the biophysical properties of supporting cells with hair cell-like characteristics and hair cells with immature hair bundles to determine how the complement of individual conductances in hair cell phenotypes are expressed and altered during hair cell development.

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.

Publications, Presentations, and Other Accomplishments:

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Effect of Skeletal Unloading on Bone Formation

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Students Funded Under Research: 0

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-1), 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-1 mRNA levels increase during skeletal unloading, suggesting that IGF-1 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-1 in vivo and in vitro. Finally, we will determine the ability of cell loading to stimulate bone cell responsiveness to GH and IGF-1 using both the Flexercell system and low speed centrifugation to load the cells then evaluating 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.

Our major effort in 1995 was devoted to determining the nature of the resistance to growth hormone caused by skeletal unloading. We made the following observations. Young hypophysectomized (HPX) rats were either skeletally unloaded (tail suspension model) or normally loaded and treated with 0,

50ug/day or 500ug/day growth hormone for 8 days. This treatment restored overall growth in all animals as assessed by gain in body weight. Growth hormone also reduced the overall reduction in bone formation as assessed by 45Ca and 3H proline incorporation induced by skeletal unloading. However, not all regions of the bone responded to growth hormone in the tail suspended animals. In particular, in the tail suspended rats growth hormone did not stimulate bone formation as assessed by double label tetracycline in the tibiofibular junction (periosteal bone formation) or increase trabecular bone volume in the proximal tibial metaphysis. The normally loaded rats responded appropriately. To determine the mechanism of this resistance to growth hormone we evaluated the mRNA levels of genes in bone known to be regulated by growth hormone. We found that skeletal unloading did not interfere with the ability of growth hormone to increase the mRNA levels of insulin like growth factor 1 (IGF-1), osteocalcin, collagen, or alkaline phosphatase. In fact, skeletal unloading appeared to increase the response of these transcripts to GH. Thus, the resistance to growth hormone (and IGF-1 demonstrated previously) occurs subsequent to the gene regulating actions of this hormone. We are currently establishing in vitro models by which we can study the mechanisms involved in this resistance at a more mechanistic level.

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 rationale drug therapy to prevent or reverse the loss.

Publications, Presentations, and Other Accomplishments:

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Plasmadesmata and the Control of Gravitropism

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FY 1995 Funding: $0

Students Funded Under Research: 4

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.

Four sets of 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 polar auxin transport is concentrated in amyloplast-containing cells. The location of the cells involved in polar auxin transport in pea epicotyls will be examined by treating the epicotyls with (3H)IAA or (3H)n3-IAA, followed by treatment with or without a polar auxin transport inhibitor which will cause a concentration of auxin in cells involved in polar transport. Autoradiography following tissue printing or photolysis will permit the identification of the cells in which IAA is concentrated. The final prediction is that when small fluorescent dyes are

injected into statocytes of pea epicotyls, their movement will be preferentially in a horizontal direction, regardless of the orientation of the tissue, while when injected into a cortical cell the movement will be equal in both directions and insensitive to gravity. A major difficulty in these experiments is that it requires the use of thin epicotyl slices. As an alternative, intact Arabidopsis hypocotyls will be tested to see if dye coupling experiments can be carried out successfully with this system.

Two major hypotheses were proposed in this task. Progress has been made in testing both of them. The first hypothesis is that in gravisensitive cells the direction of the gravity vector will influence the conductance of plasmodesmata in the lateral and longitudinal directions. Implicit in this hypothesis is the dogma that plasmodesmata normally have a size exclusion limit of about 800 Da (i.e., allow molecules of less than 800 Da to move from cell to cell). It was proposed that small molecular weight dyes such as Lucifer Yellow (LY) or carboxyfluorescine (CF) would move from cell-to-cell in a horizontal direction, but not vertically. This hypothesis has been tested in two ways. The first was to microinject LY or CF into epidermal or cortical cells of the Avena coleoptile. This tissue was chosen because the majority of its cells contain amyloplasts, and are therefore believed to be gravisensitive. The injection experiments have clearly shown that either dye injected into the cytoplasm of epidermal cells remains in those cells and does not move in any direction. When injected into subepidermal cells, however, there can be a slow movement into other subepidermal cells, and into some cortical cells as well. When injected into cortical cells, LY normally fails to move in any direction. A second approach was to incubate Avena coleoptile sections in carboxyfluorescine diacetate (CDFA), which gets taken up into cells and hydrolyzed into CF. CF will not cross membranes, with the result that it can move from cell to cell only through the plasmodesmata. To facilitate entry of the CFDA, part of the epidermis was removed from a region near the apex of the coleoptile. The results showed that CF did not move in any direction from epidermal cells. Movement in the longitudinal direction was primarily in the vascular bundle and in the subepidermal cells. Movement in the lateral direction was limited in vertical coleoptiles, with only the supepidermal cells and the small vascular parenchyma cells receiving CF. When orientated horizontally, however, there was now movement of dye from subepidermal cells into the main cortical cells.

The results, then, indicate that the dogma about the size exclusion limit for coleoptile cells is not correct. The size exclusion limit must be less than 376 Da for most of the plasmodesmata. Epidermal cells appear to be totally isolated symplastically from neighboring cells. Cortical cells are coupled with other cells only when the gravity vector is displaced from the vertical. Only the subepidermal cells are coupled to neighboring cells, and primarily to other subepidermal cells in the vertical direction. It is apparent that the original hypothesis is not supported by the data, and that gravity does not alter the direction of the polar auxin transport pathway by changing the conductance of plasmodesmata. The second hypothesis was that gravisensitive cells, but not gravi-insensitive cells will have a standing gradient of cytoplasmic calcium, with higher levels of calcium on the lower side. To test this, Avena coleoptile cells (gravisensitive) or mesocotyl cells (gravity insensitive) were loaded with the AM ester of Calcium Green-1 (CaG), a calcium-sensitive fluorescent dye. Since CaG cannot be ratioed, and it is essential to be able to ratio calcium sensitive to calcium insensitive fluorescence, the cells were also loaded with SNARF, a pH sensitive dye. The advantage of using SNARF is that it can be used to measure cytoplasmic pH, since it can be ratioed at a pH-sensitive and pH-insensitive wavelength. Confocal microscopy was used to examine the pH and calcium levels, using the ratio technique. The results showed that the cytoplasmic pH of the cells was constant in both type of cells. However, the cytoplasmic calcium appears to be higher at the bottom of coleoptile cells, which are gravisensitive, but constant in mesocotyl cells which cannot perceive gravity. Thus the results are consistent with the hypothesis.

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 have focused on one of two systems -mature leaf mesophyll cells, and phloem companion cells 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 most occur. The results of my work may point the way to future research which will provide answers to these questions.

Publications, Presentations, and Other Accomplishments:

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Basic Gravitational Reflexes in the Larval Frog

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Students Funded Under Research: 0

Task Description:

This investigation is directed towards understanding how the nervous system of the larval amphibian processes gravitational information. This study involves predominantly electrophysiology of the isolated, alert (forebrain removed) tadpole (*Rana catesbeiana*-bullfrog) head, which will survive for several days in vitro. The focus of the experiments is threefold: 1) to understand from recordings of units in the whole trochlear nerve how static and dynamic gravitational stimuli are processed by the nervous system; 2) to localize neuronal centers responsible for this processing through reversible, iontophoretic, pharmacological ablation of these centers, while maintaining trochlear nerve recordings; and 3) to record intracellularly from individual neurons within these centers in order to define the single neuron's role in the overall processing of the center. This study will provide information on the mechanisms by which a primitive vertebrate, with powerful gravitational reflexes, processes these reflexes. Such information will elucidate on an elementary level a skeletal framework of organization, upon which the more elaborate reflexes of higher vertebrates may be constructed.

A computer-driven mechanically-controlled tilt table was developed allowing precise tilt of the isolated tadpole head. Extracellular spike potentials were recorded from the trochlear nerve utilizing suction electrodes. The frequency of these potentials was increased with head up tilts and decreased with head down tilts. These recordings were digitized at 50 kHz and stored in a computer. The spike potentials were detected by a computer algorithm developed by the principal investigator. Larger amplitude spike potentials were more sensitive to tilt velocity, while the smaller amplitude potentials were more sensitive to tilt position. Modulation of the firing rates of the motoneurons was found at velocities as low as 0.023 degrees per second, suggesting that these responses were due to otolithic inputs.

Investigations of the frog hair cell-afferent fiber synapse in the three neuronal vestibulo-ocular reflex arcs explored the sensitivity of synaptic transmission to alterations in the concentrations of extracellular cations. Increases in the extracellular concentration of K^+ ions resulted in a large increase in the frequency of occurrence of EPSPs without significantly affecting their amplitudes, suggesting that small depolarizations of the hair cells result in large increases in transmitter release. Increases in the extracellular concentrations of Ca^{2+} ions decreased both the frequency of occurrence and the amplitudes of the EPSPs, while decreases in the extracellular concentrations of Ca^{2+} ions had reciprocal effects. These findings indicated that Ca^{2+} acts presynaptically to reduce transmitter release (although its entry into the hair cells is required for release to occur) and postsynaptically to block cationic entry

into the afferent, thereby reducing the size of the EPSP. Decreases in the extracellular concentration of Na^+ resulted in a decrease in EPSP amplitudes without significantly affecting the frequency of occurrence of the EPSPs, suggesting that the subsynaptic receptor on the afferent opens channels permeable to Na^+.

More recent experiments have involved investigations of the hair cell-afferent fiber synapse in the isolated turtle inner ear. Extracellular recordings from afferents show that some are spontaneously active at rest. Bath applications of glutamate or aspartate reversibly increase these firing rates. Bath application of kynurenic acid (a competitive antagonist of acidic amino acids such as glutamate) reduces both the spontaneous activity of the afferents and the increases in activity due to glutamate or aspartate, suggesting that a hair cell transmitter in the turtle could be glutamate or a similar compound.

The turtle inner ear possesses the two types of hair cells found in humans and other mammals, while only one type of hair cell is found in the frogs and tadpoles. The turtle inner ear neuropils have been investigated at the electron microscopic level to ascertain the types of synapses formed as a consequence of having this additional hair cell type. Synapses are found between type II hair cells and afferents and between efferents and type II hair cells as has been reported in frogs. In addition, type I hair cells are found virtually surrounded by calyceal afferents. Synapses are found between the type I hair cells and the calyx as well as between type II hair cells and the outer calyceal face. Efferents also contact the calyx directly. This synaptic arrangement found in the turtle is similar to that reported in mammals.

The finding that trochlear motoneuronal activity in the tadpole was sensitive to velocities as slow as 0.023 degrees per second indicates that even a vertebrate, as primitive as is the tadpole, has in the ear an extremely sensitive monitor of gravity. Modulation of the gravitational field that accompanies space flight then has a pronounced effect upon vestibular reflexes and gaze stabilization.

The marked sensitivity of the synaptic transmission between hair cells and afferents to small changes in extracellular cationic concentrations emphasizes the need for strict homeostatic regulation of these concentrations in order to maintain reliable inner ear function. The lack of such regulation could lead to significant inner ear malfunction and disorder. The finding that glutamate is likely to be the vestibular hair cell transmitter in the turtle as well as in the frog, suggests it may also be the transmitter in humans and other mammals. Known excitoxic effects of glutamate could then manifest themselves in the ear with over stimulation of the hair cells. The similar synaptic arrangements between vestibular hair cells, afferents, and efferents between turtles and mammals suggests that there are basic functional similarities as well.

Publications, Presentations, and Other Accomplishments:

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Effects of Silver and Other Metals on the Cytoskeleton

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Expiration: 2/98

FY 1995 Funding: $122,803

Students Funded Under Research: 3

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.

We had determined previously that addition of $AgNO_3$ to sea water had a major effect on the cellular shape changes of the fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta*, an effect that at the ultrastructural level was correlated with the presence intracellularly of an unusually large number of microtubules. These data left open the possibility that some or all of the cellular effects arose from the presence of impurities in the $AgNO_3$, rather than from the Ag^+ itself. To address that possibility, we generated Ag^+ by electrolysis of pure Ag^0 wire in sea water. The result was that the fertilized eggs were triggered to display all the same responses as if we had added $AgNO_3$.

We have now determined that Ag^+ may not be the only heavy metal ion to generate the characteristic cellular shape change in these cells. A narrow range of Cu^{2+} concentrations also now has been observed to cause the same cellular effects (although effects on microtubule distributions have not been determined). We have determined that gadolinium ion (Gd^{3+}) does not cause the same effects on cellular shape change as does Ag^+. Heavy metal ions, such as Ag^+, are thought to affect cellular functions by binding to proteins via a cage of three (3) sulfhydryl groups (^-SH). We asked if fertilized *Ilyanassa* eggs would show responses that mimicked those to Ag^+ if ^-SH groups were cross-linked instead in groups of two (2) by homobifunctional, sulfhydryl-specific crosslinking reagents - all in the absence of Ag^+. One such agent was identified that produced effects on cellular shape change that mimicked the effects of Ag^+. These data suggest that, however Ag^+ is affecting these cells, the mechanism involves ^-SH

groups. In the future, we must devise experiments to determine which of the Ag^+- effects observed on the cellular shape changes and on the cytoskeleton of these fertilized snail eggs arise from reactions of Ag^+ with molecules on the cell surface and which ones arise from reactions of Ag^+ with molecules inside the cell. Our observation that Cu^{2+} causes a set of cellular shape changes that closely resemble those caused by Ag^+ raises the possibility that other heavy metal ions, each in a narrow concentration range, may cause similar effects to those of Ag^+. Whether Cu^{2+}, or any other heavy metal ion, causes stabilization of microtubules to the same extent as Ag^+ remains to be determined.

Silver metal (Ag^o) and ions (Ag^+) 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 micro-organisms 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^o in the skin and mucous membranes which remains permanently as grey depositions (Ag^o 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 above. Our research represents a focused attempt to understand the molecular mechanism(s) involved in the toxic effects of Ag^+ on animal cells.

Publications, Presentations, and Other Accomplishments:

Conrad, A.H., A.P.Stephens, A.Q.Paulsen, S.S.Schwarting, and G.W.Conrad. "Effects of silver ions(Ag^+) on contractile ring function and microtubule dynamics during first cleavage in *Ilyanassa obsoleta*." Cell Motility & Cytoskel., 27, 117-132 (1994).

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Mechanisms of Differential Growth During Stem Gravitropism

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Funding:

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Students Funded Under Research: 2

Task Description:

Gravitropism offers us a valuable opportunity to learn about the interaction of gravity with growth processes and to gain insight into the cellular, physical and molecular processes that contribute to and govern plant growth. Prior work in my laboratory has shown that the gravitropic response of young cucumber stems involves a rapid and complicated alteration of growth on both sides of the stem. Cells separated only by the diameter of the stem (1.5mm) respond in opposite fashion, for the cells on the upper surface of a horizontal stem cease expansion altogether (at least transiently) whereas the cells on the lower surface double their growth rate. Our biophysical studies have shown that turgor is only passively altered during the response and that the control of growth is exerted entirely by a change in the wall relaxation properties of the wall. Although wall calcium has been postulated to be an important regulator of wall properties during gravitropism, our current work indicates that (a) it is not significantly redistributed during the initial stages of the growth response, and (b) calcium is not an effective inhibitor of cell expansion in cucumber stems (unlike some other plant tissues).

This proposal outlines a series of studies to evaluate the significance of wall pH, wall synthesis, and wall enzyme activities during the cucumber gravitropic response. Wall pH will be studied by the use of pH microelectrodes. In addition, a novel optical technique for pH measurement will be used to confirm the pH microelectrode results and to extend wall-pH measurements beyond current technological limits. Wall synthesis will be studied by microinjecting radio-labeled polysaccharide precursors into the apoplast of the two sides of the gravitroping stem. Incorporation into the major wall carbohydrates will be studied to determine whether the wall-growth changes that cause gravitropism are associated with changes in wall synthesis. Wall enzyme activities will be examined by studying endogenous wall autolytic activities, using a new method of anion-exchange chromatography and pulsed amperometric detection of sugars released by the cell walls. The ability of walls to undergo enzyme-mediated extension (creep) will be examined. Finally, wall enzymes will be studied to determine whether they are activated or inactivated by post-translational modifications, including thiol oxidation/reduction or changes in phosphorylation. The results will provide new information about the mechanism(s) by which the gravity modulates the wall-growth behavior of plant cells, and will test current hypotheses about the fundamental processes underlying plant growth.

Treatment of cucumber hypocotyls with 1 uM auxin leads to a change in the redox state of the cell wall, such that wall protein sulfhydryls become more reduced. This conclusion was established by labeling tissues (with or without auxin pretreatment) using an impermeant fluorescent thiol reagent. Wall-bound proteins were then extracted with 1 M NaCl. The protein-associated fluorescence doubled in the auxin-treated samples, yet the total amount of protein remained little affected. This result is important because it shows that auxin can cause changes in the thiol redox state of wall proteins. This fact, in combination with the observation that expansin activity is stimulated by thiol reducing reagents, suggests that a part of auxin action and gravitropism may involve control of wall redox state. We are now testing for asymmetries in wall redox potential during gravitropism.

We purified wall extension proteins from plant cell walls by HPLC and related methods in combination with our wall extension reconstitution assay. A wall protein antigenically related to cucumber expansin-29 can be extracted from oat coleoptile walls, tomato leaves and celery petioles. The single active fraction contained a single major protein band of 29 kD. In Western blots this protein was recognized by an antibody raised against cucumber expansin (the 29 kD form). Thus, expansins are found in walls from grass and other tissues, and may be ubiquitous components of plant cell walls. This implies that the biochemical mechanism for acid-induced wall expansin is common to these distantly-related plants. Both dicot and grass shoots are thought to respond gravitropically via an auxin-induced differential acidification of the wall space; we postulate that expansins are the wall protein factors that translate a wall pH asymmetry into a wall expansion asymmetry. Exogenous applications of expansins (from cucumber hypocotyls) leads to enhanced elongation of excised *Arabidopsis* hypocotyls. This is the first demonstration that expansins can enhance cell elongation in living cells. Comparison of wall autolytic activities under various conditions with long-term extension ("creep") activities of isolated walls under the same conditions shows that wall autolysis is not essential for wall creep. Similarly, exogenous application of cellulases or pectinases does not induce wall creep. On the other hand, a brief treatment of walls with pectinases or cellulases makes the walls more susceptible to the action of exogenous expansins. This last finding shows that wall enzymes that hydrolyze the wall matrix can augment the expansin-mediated wall extension, but cannot by themselves lead to sustained wall extension.

For multicellular organisms living on Earth, gravity is clearly important for processes such as long-distance fluid circulation, weight support, convective flows of gases and liquids, orientation and balance, and some aspects of growth polarity. Successful adaptation of plants, animals, and humans to life in space for long periods, perhaps even permanently, will require a fundamental knowledge of the physiological and developmental processes that are sensitive to conditions peculiar to space, such as reduced gravity. In plants, gravity is particularly important because it provides a major environmental cue directing the growth of stems, roots and (in some species) leaves. Studies of gravitropic responses should lead us to greater insight into the interactions of gravity with growth processes, and better understanding of the cellular and molecular mechanisms underlying signal transduction and control of plant growth. Such knowledge is likely to lead to advances in plant production in space and agriculture on Earth. At present, 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 that mediate the acid-growth mechanism. 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.

Publications, Presentations, and Other Accomplishments:

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Gravity-Sensitive Period During Frog Oocyte Maturation

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Solicitation:

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FY 1995 Funding: $50,000

Students Funded Under Research: 1

Task Description:

The unfertilized frog egg appears to be radially symmetric about its animal-vegetal (AV) axis. Establishment of bilateral symmetry --dorsal-ventral (DV) axis specification-- requires a 30° rotation of the vegetal yolk mass relative to the egg surface during the first cell cycle. The direction of this rotation reliably determines the DV axis orientation, and usually reflects the position of sperm entry (SEP). Thus, the SEP is often used to predict the plane of bilateral symmetry. However, the spatial relationship between the SEP and the DV axis is actually quite variable, suggesting that cues other than the SEP may also act on the rotation mechanism. One well-known external influence is gravity: when fertilized eggs are tilted from their usual orientation, gravity-driven internal rearrangements result in rotation directions different than that specified by the SEP. Endogenous cues may also be present in the unfertilized egg. For example, parthenogenetically activated eggs exhibit a normal rotation, even though they have not been fertilized. We observed that eggs of the frog *Xenopus laevis* tilted 90° off-axis during *in vitro* maturation do not have true radial symmetry. The site of polar body extrusion -- the maturation spot (MS) -- appears about 15° from the center of the animal hemisphere, closest to the point of the equator that was upmost during maturation. When such eggs are parthenogenetically activated, the vegetal yolk mass rotates toward this point. This result is biologically relevant, because most spawned eggs will likewise have experienced an off-axis orientation during maturation as they travel down the narrow, convoluted oviduct. Direct measurement reveals that, contrary to expectation, the MS of normal spawned eggs is also eccentrically located by about 15o, suggesting that off-axis influences also occur under *in vivo* conditions. As in oocytes matured *in vitro*, the yolk mass of spawned eggs rotates toward the MS, confirming the idea of an endogenous cue in the egg. The goal of the proposed project has been to identify and characterize the gravity-sensitive cellular mechanism by which frog oocytes receive this cue during maturation, and to learn its developmental significance.

The long-term goal of this project is to characterize and identify the gravity-sensitive cellular mechanism by which *Xenopus* oocytes direct the assembly of the cytoskeletal machinery used for cortical rotation. This year we have continued work on the project's specific aims, and have begun following up an interesting new lead stemming from the proposed work. We recently discovered a heretofore undescribed microtubule-containing structure in cleavage furrows in *Xenopus* eggs. Consistent with the project's long-term goal of learning the role of cytoplasmic rearrangements in

embryonic development, we have been investigating the function of this structure, and are specifically addressing the hypothesis that the microtubules serve to transport membrane vesicles toward the furrow's leading edge. This finding is consistent with the recent observation that furrow placement is sensitive to the magnitude of the gravity vector. We have established that the furrow-associated microtubules are involved in delivery of membrane vesicles to the advancing furrow.

We have also demonstrated a strong correlation between maturation spot position and yolk mass rotation direction in activated eggs, and showed that both of these features are strongly influenced by an oocyte's orientation during maturation.

In addition, in fertilized eggs, the direction of yolk mass rotation is detectably influenced by this gravity-based cue, acting in cooperation with the sperm aster. Because this rotation's direction ultimately establishes the orientation of the dorsal-ventral axis, the wide variability in the relationship between the SEP and the dorsal side probably stems from the random gravitational orientations experienced by individual oocytes during maturation. We have been testing three potential mechanisms by which gravity could cue the rotation direction: a) that the cortical cytoskeleton of the maturing egg becomes entrained in a way that promotes assembly of parallel microtubules in the vegetal cortex; b) that the yolk mass undergoes significant shape changes during maturation resulting in a volumetric shift, driven by buoyant density differences allowed to equilibrate when the cytoskeleton disassembles in the first cell cycle; and c) that a microtubule organizer, perhaps one associated with the female pronucleus, becomes displaced from a concentric, axial location.

To address the possibility that the cortex becomes functionally strained during maturation, we have been using whole-mount immunohistochemical methods and confocal microscopy to examine features of the cortex following *in vitro* maturation in off-axis orientations. Thus far we have not detected any appreciable alignment of cortical cytokeratin filaments following maturation. In recent discussions with B. Rowning (Gerhart laboratory, UC Berkeley), we learned that actin microfilaments may be playing a role in the microtubule-dependent rotation mechanism. The interplay between microfilaments and microtubules is not yet understood, so it will be important to explore this new avenue. Earlier, we had proposed using an endoplasmic reticulum-specific fluorescent lipid dye, Di(IC18)3 to study endoplasmic reticulum (ER) organization as it relates to the gravity-sensitive step. However, we learned that the dye cannot be loaded into the endoplasmic reticulum in the short time frame available during maturation experiments to adequately visualize individual ER cisternae with the confocal microscope. Our current approach to this problem is to use wholemount immunocytochemistry.

To address the possibility that the yolk mass itself has undergone a significant shape change that imparts an early rotation-initiating bias, we have developed confocal microscopic methods to examine cleared whole-mounted specimens, taking advantage of the abundant autofluorescence of yolk platelets (Brown et al., 1994; Denegre and Danilchik, 1994). The maturation spot location serves as a permanent indicator of the orientation that oocytes were in during maturation. After bleaching, the pigment becomes slightly autofluorescent, so the maturation spot's location, the animal hemisphere's pigmented cortex, and the location and shape of the vegetal yolk mass can all be related in single midsagittal and equatorial optical sections and results compared between oocytes held upright or tipped off axis during maturation. This analysis is ongoing. Dr. Steve Black (Reed College) is spending his sabbatical year in my laboratory; we plan to use the above-described approach to study yolk mass rearrangements in eggs centrifuged to produce conjoined twins. This work should produce a large collection of data which will enable us to learn details of yolk mass rearrangement and its developmental role before and during the rotation.

To address the possibility that the microtubule organizer that expands following parthenogenetic activation of the egg becomes eccentrically located during maturation, we have used confocal whole-mount immunocytochemical analyses to examine its position relative to the maturation spot in eggs manipulated to undergo *in vitro* maturation in various orientations. What emerges from this study is

that the uncleaved egg does not have a conventional centrosomal microtubule organizer. Rather, there is a broad, quite amorphous region in the center of the animal hemisphere from which microtubules emanate in roughly radial arrays. Thus, although the idea of a specific centrosome-like microtubule-organizing cell center that we had hypothesized might displace during maturation is no longer valid, it still appears likely that the amorphous microtubule-nucleating zone does shift considerably in response to off-axis gravitational force during maturation. From the information we have gathered thus far, this remains the most plausible scenario for explaining the gravity-responsive phenomenon.

While carrying out whole mount immunocytochemical analyses for the above experiments, we noticed the presence of large numbers of radially arranged microtubules in cleavage furrows. Realizing that such structures have never been described in any animal cell, we began a side project to examine their distribution and potential functions in early *Xenopus* development. This was deemed relevant to the broader goals of the project, given that furrow placement has been recently shown to be sensitive to gravity. We have established that the furrow-associated microtubules are involved in delivery of membrane vesicles to the advancing furrow, and a manuscript is in preparation.

This research addressed a basic problem in Developmental Biology: how have large cells, such as fertilized eggs and early embryos, adapted to developing in a gravitational field. Before a fertilized egg divides to produce a multicellular embryo, its contents are subject to potential rearrangement via local buoyant density differences. Some systems are very sensitive to disturbances of their gravitational environment; others are totally insensitive, and some apparently utilize this force during normal development. One well-studied embryonic system in which the gravity vector has a developmental consequence is that of the frog egg, which carries out a dramatic rearrangement of its contents during the first cell cycle following fertilization. Brief reorientation profoundly disturbs the pattern of embryonic development. We observed a gravity sensitive rearrangement in the egg's contents during ovulation, a brief period when the egg's internal structure (the cytoskeleton) disassembles and then reassembles, and now know more about how the cortical cytoskeleton organizes relative to the gravity vector during the first cell cycle. At the time we embarked on this project, it was not known whether eggs matured and fertilized in microgravity could develop normally, so the egg's potential entrainment by gravity during ovulation was a technical concern. Since then, the Spacelab J mission was conducted and the work of Souza and colleagues has revealed that normal development can proceed in microgravity.

Publications, Presentations, and Other Accomplishments:

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Yost, H.J., C.R. Phillips, J.L. Boore, J. Bertman, B.Whalon and M.V. Danilchik "Relocation of mitochondria to the prospective dorsal midline during *Xenopus* embryogenesis." Developmental Biology, 170, 83-90 (1995).

Mechanical Modulation of Striated Muscle Phenotype

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Funding:

Project Identification: 199-40-27-13
Solicitation:
Initial Funding Date: 4/95
Expiration: 3/96
FY 1995 Funding: $61,109
Students Funded Under Research: 0

Task Description:

Alterations in work are documented to modulate the size of the heart and skeletal muscle as well as the organization of the contractile apparatus within their resident myocytes/muscle fibers. The long term goal of this project is to document the role of gravity in regulating the striated muscle cell phenotype. The present project will employ cultured adult cardiac myocytes and skeletal muscle myotubes to evaluate the role of mechanical load in regulating the assembly and disassembly of the contractile apparatus and determine whether specific cytoskeletal proteins are crucial to the maintenance of its structure and contractile properties in a simulated microgravity environment. Preliminary observations indicate that the myofibrillar apparatus is disassembled in non-beating (i.e., mechanically unloaded) heart cells and the cytoskeletal proteins, alpha-actinin, desmin, and viniculin, which are believed to link the myofibril to the sarcolemma and stabilize its structure, lose their association with the dedifferentiated contractile elements. Electrically depolarizing the cells activated beating, alters mechanical load and promotes the reassembly of the contractile apparatus. The hypothesis to be tested is that gravitational forces alter the mechanical load on the heart and skeletal muscle and modulate myofibrillar-cytoskeletal interactions which, in turn, regulate the organization of the contractile apparatus and its contractile properties. Distribution of cytoskeletal and contractile proteins will be evaluated by immunofluorescence, confocal microscopy and immunogold electron microscopy. Cytoskeletal protein turnover and atrial natriuretic factor (ANF) synthesis and secretion will be monitored in these cultured myocyte/myotube preparations where mechanical load can be carefully controlled. Defining how mechanical load alters these cytoskeletal-myofibrillar interactions and ANF secretion will provide insight into the subcellular mechanisms that modulate the distribution and turnover of those proteins believed to stabilize the contractile apparatus of the cardiac myocyte and the skeletal muscle fiber in a microgravity environment where gravitational forces are markedly reduced and mechanical work is diminished correspondingly.

In the past year our efforts have focused on monitoring how changes in mechanical load influence the expression of contractile/cytoskeletal genes and the turnover of contractile proteins in cultured adult heart cells. Cardiomycrytes were either passively stretched or field stimulated at frequencies ranging from 0.5 to 4 Hz. The expression of actin and myosin mRNAs was elevated significantly within 24 hours and the fractional rate of actin and myosin synthesis was increased approximately 50%. Associated with these changes in gene transcription and contractile protein turnover, the assembly of

these nascent proteins into new myofibrils appeared to increase in proportion to changes in mechanical load. As a result of these experiments, our laboratory is attempting to identify the signal transduction pathways that regulate gene expression, protein turnover, and myofibrillar assembly. The long term goal of this investigation is to elucidate how physical force (i.e. contraction) activates specific chemical pathways that promote striated muscle growth.

Our present results support the contention that the amount of force generated by striated muscle influences the steady-state levels of contractile gene expression in both cardiac and skeletal muscle. Defining the regulatory pathways that transduce physiomechanical work into chemical mediators of gene expression, contractile protein turnover and myofibrillar assembly will provide new insights into the mechanisms that regulate the growth/atrophy of striated muscle on Earth or in a microgravity environment. Identifying the rate limiting elements of these signal transduction pathways offers an opportunity to precisely control muscle growth. Currently we are exploring how a variety of growth factors and catecholamines modulate these activities. Defining how changes in mechanical load affects neurohumoral activation may have important consequences on the evolution of physiological and pathophysiological cardiac hypertrophy.

Publications, Presentations, and Other Accomplishments:

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Otolith-Canal Convergence in Vestibular Nuclei Neurons

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Funding:

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Students Funded Under Research: 1

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 will, for the first time, determine 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 my laboratory, the effects of convergence of information from linear to angular acceleration receptors onto single vestibular nuclei neurons will be determined.

The initial phases of the current project have addressed the type and quality of information provided by the peripheral otolith system in birds. The responses of primary afferent fibers in pigeons were obtained to linear translation stimuli in an Earth horizontal plane, with the animal's head oriented at different positions relative to the stimulus axis. The orientation positions included static placements every 15° on the compass, with 90° being stimulation along the interaural axis and 0° being stimulation along the naso-occipital axis. The responses from these positions were then used to determine a maximum response direction for each otolith afferent fiber. Results from 38 afferents obtained to date show that most afferents have directions of maximum sensitivity that are directed out the opposite ear and lie in the horizontal head plane. However, about 20% of the afferents have maximum sensitivity directions directed out the ipsilateral ear. These response vectors coincide well with the known morphological polarizations of hair cell stereocilia on the utricular maculae. Thus, the utricular otolith afferents are most sensitive to side-to-side head movements, or small head tilts away from vertical. In addition, for approximately half of the recorded afferents, different frequencies of linear translation ranging between 0.2 to 10 Hz(0.2g) were also delivered. The results from these tests show that otolith afferents in pigeons have a very high gain (compared to land based mammals) to small accelerations, with responses increasing as stimulus frequency increased. Response phases remained constant across different stimulus frequencies.

These response properties of otolith afferents will now be compared to the responses of central vestibular neurons using identical stimulus protocols. 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 50% 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.

Publications, Presentations, and Other Accomplishments:

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Modulation of Bone Remodeling via Mechanosensitive Channels

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Funding:

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Students Funded Under Research: 0

Task Description:

We have characterized a mechanosensitive channel (SA-cat) in osteoblasts which we propose is the signal transducer for converting physical strain into osteogenic responses. In the studies outlined here, we plan to apply chronic, intermittent mechanical strain (CMS) to osteoblasts and osteoblast-like cells, in vitro, in conjunction with patch clamp analyses and cellular and molecular techniques to examine the role of SA-cat channel in 1) the osteoblastic response to varied magnitudes and frequencies of CMS, 2) the increase in intracellular Ca^{2+} in response to acute and chronic strain, 3) the interaction of CMS and hormonal stimulation on osteoblastic function and 4) the relationship of mechanotransduction with the extracellular matrix-integrin-cytoskeletal axis. These studies will provide insight into the anabolic effects of mechanical stimulation which become important in light of the rapid loss of bone incurred by astronauts on extended space flights.

Significant progress has been made in two areas of this grant, a) modulation and identification of the mechanosensitive channel (SA-cat) and b) expression of the matrix proteins in response to chronic mechanical strain. We have demonstrated that CMS modulates SA-cat channel activity by increasing single channel activity, including spontaneous activity, single channel conductance, and stretch sensitivity. These modifications would increase the likelihood that the SA-cat channel would respond to additional mechanical stimulus and would permit more ions to traverse the channel. Probably the most exciting advancement made in this proposal was the observation that antisense oligodeoxynucleotides developed against an isoform of the L-type, dihydropyridine-sensitive, calcium channel would abolish the functional expression of the SA-cat channel in osteoblasts. This would suggest that the SA-cat channel is an alternatively spliced isoform of the L-type calcium channel. We have also demonstrated that the channel responsive to mechanical strain is dependent on the extracellular matrix on which the osteoblast is grown. When osteoblasts are grown on glass coverslips, the L-type calcium channel is responsible for the intracellular calcium transient associated with mechanical strain. However, when osteoblasts are grown on type-I collagen coated tissue culture plates the intracellular calcium transient could be abolished by the addition of the antisense oligodeoxynucleotide to the SA-cat channel. These observations would indicate that the functional expression and mechanical response of channels are dictated by the substrate the cells are grown on and may be modulated through the integrin cytoskeletal axis.

We have also demonstrated that intermittent CMS increases the expression and production of the extracellular matrix proteins, type I collagen, osteocalcin, and osteopontin. Interestingly we found that the mechanically stimulated increase in these extracellular matrix proteins was independent and synergistic to vitamin D stimulation of the osteoblast. While these studies were conducted at supraphysiologic levels of mechanical strain, current studies have indicated that these extracellular matrix proteins are also stimulated by more physiologic levels of strain. We have demonstrated a magnitude dependent increase in osteopontin and type I collagen expression of strain from 2700 - 4200 mE. While these observations would suggest that the SA-cat channel is an important element in the mechanotransduction of mechanical strain into an osteoblastic response, we have not directly demonstrated the correlation between SA-cat channels and osteoblast function. We are currently examining the role of the SA-cat channel in the modulation of osteoblast function using antisense oligodeoxynucleotides during chronic mechanical strain. These studies will provide valuable insight into the mechanisms of mechanotransduction.

The mechanical environment is vital to the normal response in a number of physiologic systems, but perhaps to 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 for the transduction of mechanical stimuli into a cellular biochemical response will provide both physiologic and pharmacologic foci to attempt to provide methods to increase bone formation, a critical medical concern to the aging population. Furthermore with the possibility of conservation of mechanotransduction mechanisms in other systems, these studies could provide valuable insight into medical problems such as hypertension.

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Cellular Specificity in Arabidopsis Root Gravitropism

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Funding:

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FY 1995 Funding: $94,650
Students Funded Under Research: 5

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 the research in this proposal 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 would be 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*.

Since the initiation of funding in July 1995 we have focused on refining the software for the localized response measurements and using the improved software to identify sub-zones of response within the

distal elongation zone (DEZ) of roots of *Arabidopsis*. The software improvement have been completed and we used the new system to determine that there are at least two populations of cells with different gravitropism response characteristics within the distal elongation zone. We have now begun assembling the hardware required to manipulate seedling position in response to continuous analysis of angle of orientation of root subzones. Once this hardware is assembled we will use the system to compare gravisensing and graviresponse zones along the long axis of the apical portion of wild type roots before proceeding to analyze starchless mutants.

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.

Publications, Presentations, and Other Accomplishments:

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Transduction of the Gravity Signal in Roots of Corn

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Funding:

Project Identification: 199-40-57-26

Solicitation:

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Expiration: 3/98

FY 1995 Funding: $72,482

Students Funded Under Research: 1

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). The main focus of this proposal is to characterize regulators and substrates of this maize protein kinase. The proposed research would, for the first time, link together several steps hypothesized involved in the gravity transduction pathway in roots. Our second objective is to continue to explore the role of phytochrome in mediating root gravitropism. Our working hypothesis is that changes in the spatial kinetics of the phytochrome message will provide information on the locale and magnitude of other phytochrome-regulated (gravity-related) events within the root cap. A third objective is to continue to investigate whether specific RNAs and/or proteins are induced in the root cap following illumination. This approach will take advantage of the polymerase chain reaction technique, allowing us to amplify the small amounts of poly A+ mRNA in root caps.

During the previous year we have continued our studies on the identification and characterization of a maize root Ca^{2+}/CaM-dependent protein kinase homolog which we hypothesize is involved in light-mediated root gravitropism. For this effort we have isolated a cDNA from maize root caps. This cDNA encodes a maize homolog of mammalian calcium/calmodulin-dependent protein kinases. The maize cDNA is expressed in E. coli and the fusion protein has been purified. As predicted, this protein shows calmodulin-binding activity and this activity can be out-competed with a drug which specifically affects this class of kinases. We report these results in a paper in press in Planta.

Our current and future work will be directed to establishing a role for this kinase in gravity transduction. For this effort we have isolated a genomic clone of the kinase and are now in the process of cloning it into appropriate vectors. Following completion of this effort we will use the construct to transform both maize and Arabidoipsis plants, hoping to obtain over/underexpression and as well sense or antisense plants.

Another line of work has been to use our sequence in order to obtain so-called "knock-out" plants - that is, plants which have this gene interrupted by the insertion of a transposable element. This work was carried out last summer and we have several families of maize which appear to have this gene interrupted. During the following year we will first establish if this gene is in fact interrupted, and if so, will begin a physiological characterization of the plants. The work involves both molecular and physiological approaches and is carried out in both the field and greenhouse.

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.

Publications, Presentations, and Other Accomplishments:

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Microgravity In Vitro *Model of Bone Cells: Flow Effects*

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Funding:

Project Identification: 199-40-27-12
Solicitation:

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FY 1995 Funding: $50,000
Students Funded Under Research: 2

Task Description:

Interstitial fluid flow in bone results from pressure gradients induced by vascular and hydrostatic pressure, and mechanical loading. The flow rate is altered by increases in venous pressure in hypertension, fluid shifts which occur in bed rest and microgravity, increases in vascularization as seen during the injury-healing response, and mechanical compression and bending of bone during exercise. It is our hypothesis that interstitial fluid flow in bone serves to enhance transport of nutrients and cells, and mediates signal transduction in mechanical loading-induced and injury induced remodelling. We will focus this investigation on determining how interstitial fluid flow, or lack of it, may regulate osteoblasts and osteoclast function and modulate bone remodelling *in vitro*. The osteoblast differentiation and activation response will be assessed by measuring the gene expression of osteoblast marker proteins and oncogenes. The effect of flow on individual osteoclasts and the flow induced interaction between osteoblasts and osteoclasts will also be studied. The proposed investigation will provide an improved understanding of how interstitial fluid flow regulates bone function and remodelling, and it will also aid in understanding the bone loss observed over extended exposure to microgravity. Specifically, it will elucidate the importance of the fluid shift observed during microgravity exposure on osteoblast and osteoclast function.

Interstitial fluid flow may mediate skeletal remodelling in response to mechanical loading. Previously it has shown that fluid flow stimulates synthesis of prostaglandin E2, which may act to increase bone formation. Evidence suggests that bone resorption is also affected by mechanical loading. Since nitric oxide (NO) has been shown to mediate resorption in bone, we investigated and characterized the role of fluid shear on the release of NO in osteoblasts. Rat calvarial cells in stationary culture produce undetectable levels of NO, as determined by Greiss reaction. Fluid shear stress of 6 dyn/cm^2 increased NO release to 80 nmols/hr/mg protein. This release rate was sustained during the course of 12 hrs of exposure to flow. Cytokines (100 ng/ml TNF-a, 10 μg/ml lipopolysaccharide, and 100 U/ml interferon g) also induced NO synthesis, but only after a 12 hr lag phase where no NO was produced. After 48 hrs of cytokine treatment, 35 nmols NO/mg protein were produced. The cytokine-induced release of NO could be inhibited with dexamethasone, while that stimulated by flow could not. The stimulated production of NO in both cases was inhibited by N-amino-L-arginine, an NO synthase inhibitor. It thus appears that fluid shear stress can stimulate a constitutive isoform of NO synthase in osteoblasts to produce NO at rates much greater (10-fold) than is produced by the cytokine-inducible

NO synthase. These results suggest that skeletal interstitial fluid flow may regulate osteoclastic resorption as well as osteoblastic formation activity.

By establishing the role of interstitial fluid shear stress on bone remodeling, we can develop new devices to treat osteoporosis and bone fractures.

Publications, Presentations, and Other Accomplishments:

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Rossitti, S., J.A.Frangos, P.R.Girard and J.Bevan "Regulation of vascular tone1." Canadian Journal of Physiology Pharmacology, 73, 544-550 (1995).

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Mechanism of Phytochrome Regulation of Shoot Gravitropism in Arabidopsis

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Funding:

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Expiration: 6/97

FY 1995 Funding: $82,000

Students Funded Under Research: 3

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 my 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 of phytochrome B 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 B genes to investigate phytochrome B regulation of gravitropism in hypocotyls, roots, and in fluorescence stalks 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.

In the course of our investigations of the possible role of the Pr form of PHYB in regulating gravitropism, we discovered that the original phyB mutant line that we used in our earlier work had a second mutation that was responsible for the agravitropic phenotype that we had earlier attributed to a function of phyB. We have succeeded in genetically separating this gravity response mutation from the phyB mutation and are conducting more detailed genetic and physiological analyses of the mutant gene in the absence of PHYB. The gene is currently being mapped and efforts will be initiated to clone the gene. Populations of mutagenized seeds are being screened to identify additional alleles of the gravity mutation and other gravity response mutations. Photobiological studies on the interaction of the

phytochrome and gravity photosensory systems in *Arabidopsis* has now shown that both red and far-red light can modulate gravitropism in *Arabidopsis* seedlings. Analyses of specific phytochrome mutants showed that light-modulation of gravitropism is dependent on at least two different phytochromes, PHYA and PHYB. The response to far-red light is mediated by PHYA and the red response is mediated by PHYB. Although PHYA and PHYB can function independently to alter the gravity response, their differential responsiveness in red and far-red light provide a sensitive mechanism for altering growth orientation under different light qualities. The phytochrome-dependent effects on gravitropism are restricted to the aerial portions of the plants. We have also discovered a novel effect of phytochrome in regulating the angle of leaf orientation by affecting gravitropism. Under high red to far-red light ratios, leaves are horizontally aligned but at low red to far-red ratios the leaves are more vertical. These findings suggest that light quality within a plant canopy may determine the angle of branching and leaf arrangements and, thus, could be an important regulator of overall plant morphology. Because gravitropism research has traditionally focused on the primary stem and root of seedlings, these observations open new lines of investigation that should provide novel insights into the mechanisms by which plants coordinate the arrangement of lateral organs. Research on gravitropism in lateral organs is a new direction that we will be following.

During this past year, we discovered that when dark-grown seedlings are transferred to red or far-red light, the growth pattern of the elongating portion of the hypocotyl undergoes differential growth so that the hypocotyl curves. The curvature approaches 90 degrees after about 20 hours and seems to be due to a shift from negative gravitropism to plagiogeotropism. The curvature that develops resembles that for phototropism except that the direction is independent of the direction of the light source but dependent on the gravity vector. Recent work conducted in collaboration with Dr. W. Briggs and Dr. E. Liscum has shown that this phytochrome effect on gravitropism is an essential part of the blue-light-induced phototropic response. The evidence indicates that the phytochromes cause the curvature by altering the gravitropic response and the blue light system provides information about the direction of the light. The interaction of gravitropism, phototropism and the different phytochromes that have been identified by using various mutants accounts for many of the complex aspects of the phototropic response that have remained mysterious for decades. We will continue investigating the novel interactions uncovered during these initial studies. 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 possible higher yields.

Publications, Presentations, and Other Accomplishments:

Hangarter, R.P., J-H Kim and B. Tamot "Interaction between the phytochrome and gravity sensor-response systems in *Arabidopsis*." Keystone meeting on signal transduction systems in plants. J. Cellular Biochem, Supplement 16C, (1995).

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Kim J-H., B. Tamot and R.P.Hangarter "Interaction between the phytochrome and gravity sensory-response systems in *Arabidopsis*." 6th International Meeting on Arabidopsis Research, Madison, WI, 1995.

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Poppe C., R.P.Hangarter, R.A. Sharrock, F.Nagy, and E. Schafer. "Light-induced reduction of the gravitropic growth-orientation of seedlings of *Arabidopsis thaliana (L.) Hyenh* is a photomorphogenic response mediated synergistically by the Pfr forms of phytochrome A and B." Planta, In press, (1995).

Interaction of Light and Ethylene in Stem Gravitropism

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Funding:

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Expiration: 9/95

FY 1995 Funding: $43,000
Students Funded Under Research: 7

Task Description:

I propose to investigate the influence of red irradiation on the regulation of ethylene production during the time course of gravitropic bending in etiolated plant stems. Red-light pretreatment is known to restrict the locus of curvature and increase the extent of the counter-reaction (bending away from the vertical) in etiolated pea stems resulting in increased net curvature. This research proposes to determine the control of ethylene biosynthesis during early upward curvature and the counter-reactive phase which occurs in the later stages of gravitropic curvature of many etiolated stems. To accomplish this, the levels of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), the malonyl-ACC conjugate, and ACC oxidase activity will be compared in dark-grown and red-irradiated etiolated pea stems before and at intervals during gravitropic curvature. Tissue localization of ACC oxidase and ACC synthase will be determined by Western blots on longitudinal stem sections transferred to nitrocellulose prints. Thus, this project will establish the regulation points and tissue specific changes in the ethylene biosynthetic pathway for red-light pretreatment and gravistimulation. This information will contribute to further knowledge of the transduction mechanisms of gravistimulation and red-irradiation in etiolated stems. This study is also of interest to flight experiments in terms of identifying physiological changes associated with light and ethylene biosynthesis which may influence the kinetics and pattern of stem growth under microgravity conditions in closed environmental chambers.

The major objective of this study was to evaluate light-regulated production of the plant hormone, ethylene, during gravitropic bending in etiolated pea stems. Both red-light and ethylene alter the kinetics and locus of curvature along the stem. Previous investigations indicated that ethylene production increases during gravitropic curvature particularly after the initial phase of upward bending. To date we have successfully evaluated the biosynthetic steps of ethylene production during gravitropic curvature in the presence or absence of red-light treatment. Specifically we have answered the following questions: (1) Which regulatory step in ethylene biosynthesis is primarily affected by a change in stem orientation? (2) Which regulatory step is affected by red-light treatment? For dark-grown pea stems, the tissue level of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) increased slightly by 30 min gravistimulation and significantly by 90 min. However, red-light pretreatment (18 hours prior to experimentation) reduced overall ethylene production in dark-grown stems resulting in a decrease of ACC content paralleled by an increased conjugation of ACC during the later phases (90 to 120 min) of upward bending. Whether theses changes in ACC and conjugated ACC

levels are localized in the upper or lower portion of the curving stems was also addressed. However, no significant differences in ACC and MACC levels between upper and lower etiolated stem tissue was found in either dark grown or red-treated plants. Therefore, we concluded that ethylene biosynthesis is altered throughout the stem as a result of change in orientation and light treatment. It was also determined that the rate of ACC conversion to ethylene (ACC oxidation) does not significantly change during gravitropic bending. Since increased ACC was not due to decreased conjugation or decreased ACC oxidation, it was concluded that accumulation of ACC is primarily determined by synthesis of ACC. Therefore, we propose to focus on changes in ACC synthase transcription for the continuation of this research.

For studies involving microscopic analyses, we established protocols for quantifying gravity- or red light- induced changes in transcriptional (e.g.ACC synthase mRNA) and post translational levels (e.g., peroxidase and invertase activity) through computer imaging of tissue prints. Currently, we quantified peroxidase activity (as a model system) using microscopy and computer image analysis successfully measuring density changes along cross and longitudinal section of the stem imprinted on nitrocellulose. In tissue prints of pea stems, the vascular tissue had significantly more peroxidase activity than the cortex. Immunoblotting with anti-horseradish peroxidase (HRP) showed cross reactivity with the cortex of the stem only indicating that the vascular form of peroxidase is a different isozyme than HRP. Currently, mRNA probes for ACC synthase with colorimetric conjugates are being developed to use in this system.

This research provides new understanding of basic biological processes two ways. First, the interaction of light and ethylene production during gravitropism in plant stems is poorly understood. The primary regulatory points for this interaction are elucidated by this project. This provides an additional level of complexity to the understanding of the relationship between light and hormone production in plants. Second, this research evaluates biochemical processes by both biochemical and microscopic approaches. The traditional biochemical analyses use extracts which do not maintain tissue and cell organization but are highly quantifiable. Image analysis of tissue prints will provide a quantitative approach to microscopic examination of biochemical steps within the tissue. Additionally, modern molecular approaches (mRNA probes) will be integrated into this system.

Ethylene is a plant hormone which inhibits cellular and tissue growth and affects numerous plant developmental processes such as leaf drop, fruit ripening, flower development and gravitropism. All plants emit ethylene to some extent. Therefore, atmospheric ethylene levels can increase dramatically when plants are grown in closed environmental chambers such as those used aboard the Space Shuttle or on a space station. Understanding the changes in ethylene production and its interaction with light will provide a basis for the design of plant growth facilities in space. These facilities will require optimization of lighting and growth conditions within a relatively small space. Ethylene accumulation can be regulated biochemically at the plant level to optimize growth. For example, inhibition of ethylene will prevent growth inhibition and stimulation of ethylene will prevent spindly stem growth.

Ethylene has historically been the easiest plant hormone to use for agricultural applications. Its regulation has been used extensively in fruit ripening and tuber storage. Understanding the interaction of light and ethylene may provide further application in regulating ethylene in plants for agriculture purposes.

The combination of molecular, biochemical and microscopic analyses of this system will allow for greater interpretation of hormone responses. Tissue printing is a rapid procedure and allows numerous replicates to be easily evaluated. Thus, many tissue samples can be screened and evaluated for further study using traditionally histological techniques or molecular approaches (e.g., electrophoresis, immunoblotting, Northern blotting). Also, image analysis of tissue prints will provide quantitation of colorimetric responses making this a more powerful tool.

Publications, Presentations, and Other Accomplishments:

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Steed, C.L. and M. A. Harrison "Effect of red light on cellular growth processes during gravitropism in etiolated pea stems." Plant Physiol., 108, S-57.

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Taylor, L.K. and M.A. Harrison "Evaluation of soluble cell wall proteins during gravitropism in pea stems." West Virginia Academy of Science, 67, 36.

Taylor, L.K. and M.A. Harrison "Evaluation of soluble cell wall proteins during gravitropism in pea stems." Plant Physiol, 108, S-57.

Magnetophoretic Induction of Root Curvature

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-40-57-37

Solicitation:

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Expiration: 4/96

FY 1995 Funding: $60,000

Students Funded Under Research: 2

Task Description:

The goal of this proposal is to study the perception mechanism of the gravitropic stimulus. Various mechanisms have been proposed for gravity perception ranging from external ion currents to the more generally accepted concept of gravity induced displacement of amyloplasts. The common techniques of testing gravity sensing do not differentiate between forces acting specifically on amyloplasts and the surrounding tissue. We propose to study gravi-sensing by physically displacing amyloplasts by high gradient magnetic fields. This is possible because of the considerable difference between the susceptibility of the diamagnetic amyloplasts and other cellular components. Due to the uniformity of magnetic susceptibility of other biological material and the similarity to water other cellular components are not affected. The growth changes resulting from such magnetophoretical displacement of amyloplasts will be measured using video-microscopy that permits a simultaneous measurement of growth rate and curvature. Specific goals include determination of the range of cells capable of responding to amyloplast displacement; examining whether the response to magnetophoresis is comparable to gravity; significance of ion current in the perception of gravity; and comparison of response time to stimulation by gravity and magnetic forces. The long-term goal of this research is to study whether the gravity stimulus can be replaced by other (e.g., magnetic) stimuli.

Publications, Presentations, and Other Accomplishments:

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Blancaflor, E. and K.H.Hasenstein "Growth and microtubule orientation of maize roots subjected to osmotic stress." Internat. J. Plant Sci., 156, 794-802 (1995).

Blancaflor, E.B. and K.H. Hasenstein "Growth and Microtubule Orientation of Corn Roots after Exposure to Osmotica." AAIA.

Blancaflor, E.B. and K.H.Hasenstein "Microtubule orientation and growth of maize roots after osmotic shock." Plant Physiol., 108, 39 (1995).

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Blancaflor, Lee, Hasenstei "Probing microtubule function in root gravitropism using cytoskeletal inhibitors." IPGSA.

Hasenstein, K.H., J.S.Lee, and E.B. Blancaflor "Auxin transport and graviresponse of maize roots treated with microtubule inhibitors." ASGSB Bulletin, 9, 37 (1995).

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Kuznetsov, O.A. and K.H.Hasenstein "Particle magnetophoresis as a tool to study the gravity sensing system. Plant Physiol., 108, 136 (1995).

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Wan, Hasenstein. "Immunological detection of ABA-binding proteins in plants." IPGSA, 1995.

"Baby Machine" Analysis of Cellular Gravity Sensitivity

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Funding:

Project Identification: 199-40-27-20

Solicitation: 93-OLMSA-07

Initial Funding Date: 5/95

Expiration: 5/98

FY 1995 Funding: $95,854

Students Funded Under Research: 1

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. During the first year, the involvement of the gravity vector in the orientation of mitosis will be determined, as well as the existence of gravity-sensistive "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 overall goal of the project is to apply the "baby machine" culture technique to studies on the effects of gravity on cell growth, through analysis of effects on mitosis, growth rates, chromosome replication and segregation, and cellular senescence. The specific aims for FY95 were to design and construct modifications to the baby machine culture system for application to investigation of gravity sensitivity, and to use this modified culture system to evaluate the effects of gravity on the positioning of mitosis. To these ends, a simple culturing technique was developed. Standard culture flasks were coated with a thin layer of polyhydroxyethylmethacrylate, and then exposed to a suspension of small (ca. 4 mm-diameter) polystyrene beads. Upon addition of CHO cells to these treated flasks, the cells bound only to the beads such that upon subsequent growth and division, one daughter cell remained attached to the bead and the other was released. The flasks were oriented in different positions with respect to the gravity vector and the orientations of mitosis were observed by time-lapse videography. The video records are presently being analyzed, so only preliminary information is currently available. However, these preliminary analyses suggest that the orientation of cellular mitosis is unaffected by the gravity vector. These analyses will be completed during the current fiscal year, and the next step in the project, to measure gravity effects on growth rates and mitotic cycle times, will be initiated.

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.

Lineage Analysis of Axis Formation Under Novel Gravity

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Funding:

Project Identification: 199-40-27-22
Solicitation: 93-OLMSA-07
Initial Funding Date: 6/95
Expiration: 6/98
FY 1995 Funding: $142,147
Students Funded Under Research: 0

Task Description:

Recent intriguing work by Cooke ('86) and Neff, et al. ('93) 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. In this proposal, 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, and then 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 past half year, we started Experiment I proposed in the grant. The question asked in Experiment I was: "Is cell fate changed under novel gravitational fields and is this change responsible for the morphological changes?" The preliminary results from the work of the past 5-6 months gave the answer to the first half of the question, that is, cell fate is changed under novel gravity.

Thus far, we studied the cell fate change of all the blastomeres at the 8-cell stage and some blastomeres at the 32-cell stage in embryos subjected to 90-degree rotation before the first cleavage. We found that at the 8-cell stage, the blastomeres always contribute to the rostral-dorsal part of the tadpole and those on the bottom contribute to the caudal-ventral part of the tadpole. At the 32-cell stage the blastomeres adapt fates according to the new position. However, the complicated pattern of the fate change does not simply reflect a cytoplasmic shift after the rotation, but it may be a combined effect of activation. It is important to understand the combined effect of cytoplasmic reorganization caused by novel gravity and sperm activation. It is important to understand the combined effect of sperm activation and gravity on the cytoplasmic reorganization and fate change of the cells, which was not proposed in the original project description. We are going to spend some time in year 1 to get data in this respect.

We have not started the horizontal clinostat experiments which were proposed to start in year 1 and expected to give answers to the second half of the question asked in Experiment I. We will start these experiments soon. But from the preliminary results accomplished thus far we are expecting to see more effect of the gravitational change on the cell fate from the rotation experiments than horizontal clinostat experiments. Towards the end of the year 1 we are also going to start Experiment III as originally proposed.

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.

Publications, Presentations, and Other Accomplishments:

Huang, S., and Johnson, K. "Cleavage stage blastomeres of the *Xenopus* embryo change fate under novel gravity." ASGSB Bulletin, 9, 72, (1995).

The Effects of Microgravity on Bone Osteoblast Growth

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Funding:

Project Identification: 199-40-27-06

Solicitation: 91-OSSA-15

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Expiration: 12/95

FY 1995 Funding: $122,329

Students Funded Under Research: 2

Task Description:

One of the most serious health hazards to long term manned space flight is the loss of bone. 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 percent 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-Gravity 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 PGE2 from osteoblasts. PGE2 has been shown to increase trabecular bone formation in rats and infants, but the mechanism of action of prostaglandin E2 in stimulating osteoblast growth is unknown. A second and indirect effect of microgravity is an increase in cortisols in crew members. Urinary cortisols of crewmen increased from an average preflight value of 54±4 μg/total volume to 94±5 inflight 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 here on the 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-Gravity. 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 the glucocorticoids in bone loss. This information will help us understand glucocorticoid-induced bone loss both in space and in disease states. Our third objective is to discover new strategies to combat bone loss. This includes using glucocorticoid-blockers as well as designing new compounds that will stimulate osteoblast growth. 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-Gravity conditions could provide the preliminary information to establish medical intervention of bone loss, both in space and on Earth.

We have studied the effect of mechanical stress on bone osteoblasts in a series of eight experiments conducted over the last year. We found that osteoblasts release PGE2 when stressed, that COX-2 increases with stress and that EP receptors are the probable effector of action. Concerning the effect of glucocorticoids on bone cell growth and the regulation of gene expression, we found that levels of glucocorticoids comparable to those of astronauts in space flight inhibit early immediate gene induction in bone cells. We also found that there is co-regulation of genes c-fos, cPLA2 and COX-2 but not actin or cyclophilin, and that regulation is probably occurring through the NFKb promoter region for some of these genes. Further, we have fabricated c-fos constructs with GFP (green fluorescent protein) to enable definition of key promoter regions responsible for bone cell activation and glucocorticoid inhibition of osteoblasts. Finally, we have completed analysis and publication of the results of the first flight experiment using ground based resources reporting a decrease of PGE2 synthesis and growth of osteoblasts in space flight.

Osteoporosis is a generic term used to describe various bone diseases that are manifested by resulting in fractures of the vertebrae, wrist hip, humerus and tibia. Osteoporosis is common in older adults, in the presence of glucocorticoid excess as in Cushings syndrome and in people treated for asthma with steroids. Osteoporosis has also been noted in healthy astronauts that are in microgravity for extended duration. Our studies are concentrated on the basic mechanisms that regulate new bone growth and the relationship of growth to drugs and environment. In our ground studies we hope to find the basic signals which will increase bone growth and formation.

Asthma patients, Cushing patients and astronauts that have osteoporosis have one thing in common, an increase in glucocorticoids. After analysis of SKYLAB data, have 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 addition, 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 which is needed for bone growth. This is probably the major mechanism by which exercise augments bone growth (manuscript in preparation). 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.

Publications, Presentations, and Other Accomplishments:

Hughes-Fulford M. "G1 cell cycle arrest by dmPGA1: Up-regulation of c-fos and c-myc oncogene expression without transition into S-Phase." J. Cell Biochem., 54, 265-272 (1994).

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Hughes-Fulford, M. National Science Partnership of Girl Scouts and National Science Museums Educational Video for Girl Scouts of the USA, NY, 1994.

Lewis, ML and Hughes-Fulford, M. Cellular responses to microgravity. "Textbook for the International Space University." Edited by: Suzanne Churchill. Harvard University (in press), Chapter 3, 1995.

Mechanochemical Coupling between ECM and the Cytoskeleton

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Funding:

Project Identification: 199-40-27-15

Solicitation:

Initial Funding Date: 9/95

Expiration: 8/96

FY 1995 Funding: $ 85,000

Students Funded Under Research: 2

Task Description:

The general goal of this ground-based project is to characterize the molecular mechanism by which mechanical signals, such as those due to gravity, are transduced into changes in cell form and function. The more specific objective is to analyze the process by which mechanical forces are conveyed by extracellular matrix (ECM) molecules, transmitted across the cell surface, and transduced into a cellular response. This approach is based on the concept that cell shape, and thus the form of the cytoskeleton (CSK), depends on a dynamic balance between tensile forces that are generated within contractile microfilaments and resisted by both internal CSK struts and external ECM adhesion sites at the cell periphery. If this type of tensional integrity or "tensegrity" mechanism is used by cells, then transmembrane ECM receptors, such as integrins, may mediate mechanochemical transduction by transmitting mechanical stresses across the cell surface and thereby, altering the CSK force balance. The specific aims of this proposal are: 1) to identify molecules that mediate mechanical force transfer between ECM and the CSK, 2) to quantify changes in CSK mechanics that result from altering the balance of forces across specific transmembrane receptors, and 3) to analyze how changing this balance between inward and outward-directed forces alters CSK filament distribution and assembly.

To determine whether integrins act as mechanochemical transducers, we developed a magnetic twisting device in which controlled mechanical stresses can be applied directly to cell surface integrin receptors. Stresses are applied by twisting surface-bound ferromagnetic microbeads (5.5 μm diameter) that are coated with integrin ligands (e.g., fibronectin, antibodies, synthetic RGD-peptides). The cellular deformation that results in response to stress application is determined by simultaneously quantifying bead rotation (angular strain) using an in-line magnometer. Using this approach, we were able to demonstrate that application of torque (shear stress) to integrins resulted in a stress-dependent increase in CSK stiffness (defined as the ratio of stress to strain) which required intact microtubules and intermediate filaments as well as microfilaments. Interestingly, tensegrity models (1) containing mechanically-interdependent struts and strings that reorient globally in response to a localized stress mimicked this response. In contrast, force application to another transmembrane receptor that does not normally mediate cell-ECM adhesion (acetylated-LDL receptor) did not induce CSK stiffening. Other

integrin subtypes and cell-cell adhesion molecules (e.g., PE-CAM, ELAM) also have been found to mediate force transfer, however, to a lesser degree than integrin b1.

In these studies, transmembrane force transfer correlated with recruitment of focal adhesion complex (FAC) proteins (e.g., vinculin, talin, a-actinin) and thus, linkage of integrins to the actin CSK. In collaboration with Dr. Robert Ezzel (Mass. General Hospital), we have recently carried out studies using our magnetic bead twisting device to examine the mechanical properties of F9 cells that are missing vinculin. Importantly, vinculin-deficient cells do not stiffen in response to stress application to integrins whereas transfection of the vinculin gene restores the ability of these cells to respond to applied stress. In a separate collaboration with Dr. Thomas Kupper (Brigham & Women's Hospital), we were able to show that the cytoplasmic domain of the a2 integrin chain binds directly to F-actin in vitro. This finding of a direct physical interaction between a specific integrin subtype has been reported to exhibit a greater capacity for transferring cytoskeletal tension across the cell surface and to external ECM substrates (i.e., causing enhanced substrate contraction) in past studies with fibroblasts.

These data indicate that integrins act as cell surface mechanoreceptors and suggest that they transmit mechanical signals to the CSK via binding interactions with specific FAC proteins. Mechanochemical transduction, in turn, may be mediated simultaneously at multiple locations by force-induced CSK rearrangements that result in redistribution of associated elements of the cell's metabolic machinery. As an example: we have found that many of the chemical signaling molecules that are sensitive to ECM binding are immobilized and greatly enriched on insoluble CSK scaffolds at the site of integrin binding. We discovered this by studying intact FACs that formed within 15-30 minutes when cells were allowed to bind to magnetic microbeads coated with integrin ligands but not with acetylated-LDL. Newly formed FACs were isolated and collected for biochemical analysis using a combination of detergent extraction, sonication, dounce homogenization, and magnetic pelleting. Isolated bead complexes were greatly enriched for all FAC structural proteins as well as multiple chemical signaling molecules (e.g., c-src, pp125FAK, inositol lipid kinases, phospholipase C, Na^+/H^+ antiporter) and enzyme activities (e.g., c-src, pp125FAK, PIP kinase activities) when compared with either the whole CSK or basal cell membranes wherein actin (a general CSK marker) was relatively depleted.

In separate studies, we used the same device to investigate how ECM alters the mechanical properties of the CSK. We found that increasing the number of basal cell-ECM contacts by raising the FN coating density from 10 to 500 ng/cm^2 promoted cell spreading by 5 fold and increased CSK stiffness, apparent viscosity, and permanent deformation all by more than 2 fold, as measured in response to application maximal stress (40 dyne/cm^2) at the cell apex. When the applied stress was increased from 7 to 40 dyne/cm^2, the stiffness and apparent viscosity of the CSK increased in parallel although neither cell shape, ECM contacts, nor permanent deformation was altered. Application of the same stresses over a lower number ECM contacts using smaller beads (1.4 micro-m diameter) resulted in decreased CSK stiffness and apparent viscosity, confirming that this technique probes into the depth of the CSK and not just the cortical membrane. When magnetic measurements were carried out using cells whose membranes were disrupted and ATP stores depleted using saponin, CSK stiffness and apparent viscosity were found to rise by approximately 20% whereas permanent deformation decreased by more than half. Addition of ATP (250 micro-M) under conditions that promote CSK tension generation in membrane-permeabilized cells resulted in decreases in CSK stiffness and apparent viscosity which could be detected within 2 minutes following ATP addition, prior to any measurable change in cell size. Permanent deformation only decreased after 20 minutes, once the CSK lattice had physically contracted. Importantly, regardless of cell shape or membrane continuity, CSK stiffness increased in direct proportion to the applied stress, as predicted by tensegrity (tensional integrity) cell models. These results confirm that the effects of ECM on CSK mechanics are not due to changes in osmotic or hydrostatic pressures. Rather, ECM alters CSK stiffness and apparent viscosity by binding integrins, promoting formation of molecular links with the CSK, transmitting mechanical stresses across these linkages, and inducing structural rearrangements within a continuous, tensionally-integrated CSK lattice. In contrast, permanent deformation in the CSK appears to be more tightly coupled to cell extension and depends on both passive plasticity and dynamic remodeling events.

We have used a different experimental approach to analyze the effects of applying mechanical stresses to integrins on CSK as well as nuclear structure in intact and membrane-permeabilized cells (manuscript in preparation). Microbeads (4.5 micro m diameter) coated with different ligands were allowed to attach to receptors on the apical surface of cultured cells and then the beads were pulled using uncoated glass micropipettes which do not bind cells directly. Alternatively, glass micropipettes were coated with protein ligands and used to apply mechanical tension directly to cell surface receptors. When cells were pulled using either micropipettes or beads coated with integrin ligands, such as fibronectin or synthetic RGD-containing peptide, and simultaneously analyzed using video microscopy, coordinated changes in cell and nuclear form were observed. Immunfluorescence staining of cells fixed after mechanical perturbation revealed that actin nets realigned and formed higher orders actin bundles oriented along the axis of the applied tension field. Nuclear distortion was accompanied by internal rearrangements, including changes in nucleolar shape and orientation. In contrast, application of tension to beads coated with acetylated LDL, a ligand for another cell surface transmembrane receptor (non-integrin), did not result in any changes in intracellular structure; the beads simply detached from the cells. Structural continuity between integrins, CSK filaments, and nuclear components was confirmed by demonstration of similar effects after membrane-permeabilization and loss of soluble cytoplasmic components. These results suggest that when tension is applied to integrin receptors, it results in mechanical force transfer across a series of molecular bridges that physically interconnect the cell surface with the CSK and nucleus. Coordinated changes in structure throughout the cell and nucleus may serve to integrate chemical and mechanical signaling systems.

We have also made great progress in studies which center on analysis of microtubule (MT) regulation. Our reason for changing direction from actin and focusing on MTs for analysis of CSK polymerization was that thermodynamic analysis of MT assembly predicts that the concentration of free tubulin monomer must vary if MTs are to remain stable under different mechanical loads that result from changes in cell adhesion to the ECM. This potential mechanism for regulation of CSK structure by mechanical stresses is complicated, however, by the observation that cells have evolved an autoregulatory mechanism to dampen variations in the concentration of tubulin monomer that is available to polymerize into MTs, a process that is known as tubulin autoregulation. To determine how these seemingly contradictory regulatory mechanisms co-exist in cells, we measured changes in the masses of tubulin monomer and polymer that resulted from altering cell-ECM contacts. Primary rat hepatocytes were cultured in chemically defined medium on bacteriological petri dishes that were precoated with different densities of laminin (LM). Increasing the LM density from low to high (1 to 1000 ng/cm^2) promoted cell spreading (average projected cell area increased from 1200 to 6000 micro-m^2) and resulted in formation of a greatly extended MT network. Nevertheless, the steady-state mass of tubulin polymer was similar at 48 hours, regardless of cell shape or ECM density. In contrast, round hepatocytes on low LM contained a 3-fold higher mass of tubulin monomer when compared with spread cells on high LM. Furthermore, similar results were obtained whether LM, fibronectin, or type I collagen were used for cell attachment. Tubulin autoregulation appeared to function normally in these cells as tubulin mRNA levels and protein synthetic rates were greatly depressed in round cells that contained the highest level of free tubulin monomer. However, the rate of tubulin protein degradation also slowed by approximately 3-fold as the LM density was lowered from high to low. These results indicate that the set-point for the tubulin monomer mass in hepatocytes is regulated by the density of ECM contacts, and are consistent with a mechanism of MT regulation in which the ECM stabilizes MTs by both accepting transfer of mechanical loads and altering tubulin degradation in cells that continue to autoregulate tubulin synthesis.

Taken together, these results add additional experimental support to our working hypothesis that cells sense and react to mechanical stimuli via a tensegrity mechanism. Furthermore, we now have been able to develop a mathematical basis to explain the response of living cells to mechanical stresses, beginning with first principles, again by using the tensegrity paradigm. Future studies will attempt to combine this mathematical model with experiments involving living cells to test this architectural hypothesis directly.

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 would then occur as a result of subsequent alterations of cytoskeletal filament rearrangements 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 ECM attachment sites on the surface of the cell. If this type of tensional integrity or "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. The cell magnetometry method we developed for probing cytoskeletal mechanics in living cells also may potentially be useful as a non-invasive method for diagnosing changes in cell structure and/or contractility.

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The Effects of Gravitational Loading and Vibration on Vestibular Ontogeny

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Task Description:

The specific aim of the research is to test the working hypothesis that gravitational loading and/or seismic vibration does not alter normal development of vestibular sense organs. The research will determine whether changes occur in vestibular ontogeny when avian embryos (*Gallus domesticus*) are incubated under a 2G gravitational load or during whole-body vibration. The purpose is to evaluate whether or not these environmental factors can significantly alter the course of vestibular functional development. Vestibular afferent thresholds and activation times (response onset latencies) will be measured to evaluate the sensitivity and maturity of peripheral vestibular receptors, synapses and conducting neurons. Findings of significant changes in development will rule out the working hypothesis and suggest further that embryonic vestibular sensory experience may play some role in shaping the ontogeny of vestibular function. The specific physiological question to be addressed by the proposed research is: Does gravitational loading (2G) or whole body vibration during ontogeny significantly change vestibular compound action potential thresholds and/ or activation latencies in the chicken?

1. The adequate stimulus for vestibular responses is linear jerk (time derivative of acceleration, dg/dt): A new stimulus was tested and used to initiate in the study of avian vestibular ontogeny. Results showed that response thresholds, latencies and amplitudes are dependent upon linear jerk magnitudes (units of g/msec) and not simply acceleration amplitudes (g). These results affect all future investigations since they define the adequate and proper stimulus to be used for recordings of vestibular responses. The findings provide new insight into the neural generators of the vestibular responses as well as the characteristics of natural stimuli for gravity sensors.

2. Embryonic vestibular function: Definitive studies were completed to demonstrate that responses to pulsed linear acceleration were in fact vestibular in the embryo. Response parameters were quantitatively characterized and the data form the basis of a publication entitled: "Vestibular responses to linear jerk in the chicken embryo." These results establish the normal characteristics of embryonic responses at the 19th day of incubation.

3. Effects of substrate vibration on vestibular development: Vestibular function was tested in normal untreated animals and in animals exposed to seismic vibration during incubation through the first two weeks post hatch. Results from the two treatment groups are currently being analyzed and compared.

4. Normal vestibular ontogeny in the chicken (*Gallus domesticus*): Vestibular response thresholds, latencies and amplitudes were characterized for embryos and hatchlings between the ages of E17 and 30 days post hatch in the chick. These data form a normative data base for the developing chick and serve as a laboratory standard for future studies including those evaluating the effects of space flight, vibration and centrifugation on vestibular ontogeny.

5. Normative data base for adult quail (*Coturnix coturnix japonica*): Vestibular responses to linear acceleration pulses were characterized in adult quail for the first time. Vestibular response thresholds, latencies and amplitudes were measured using linear jerk stimuli. The ontogeny of function in quail remains to be characterized (pending funding). These data will serve as the laboratory standard for future research (effects of space flight (SLM1)).

6. Gravitational loading and vestibular ontogeny: A complete study was organized to compare vestibular function in animals incubated and hatched at 1.0G (normal), 1.2G (rotation control) and 2G. This study was initiated but could not be completed due to a malfunction in the centrifuge. Another study has been planned and will be carried out during the period of grant extension.

The results of work completed to date suggest that the gravity receptors of developing birds are dynamic in that they exhibit an increase in sensitivity in late embryos and early hatchlings. 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 1.0G 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. Studies planned will evaluate the influence of hypergravic fields on receptor function in developing animals. Another potential influence on the development of vestibular sensors is vibration. Cranial vibration may be introduced to developing embryos during space flight or centrifugation. Indeed, investigators could not rule out vibration as the cause for altered vestibular thresholds found in early space flight experiments. The current research has confirmed our working hypothesis that vibration (20dB above background) does not alter vestibular thresholds. Therefore, space flight vibration is an unlikely cause for the abnormal thresholds of birds incubated in space.

During the course of this research, we have demonstrated that gravity receptors in maturing chicks are functionally resilient and are capable of complete recovery following severe injury. The discovery of mechanisms controlling recovery could lead to new clinical strategies for the deaf and dizzy patient.

The research has clarified the relationship between head motion and the activation of neurons producing vestibular responses. Vestibular responses to pulsed linear acceleration likely reflect 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 critical that we clearly define the nature of the functional test and establish that it is, in fact, a vestibular test for all ages studied. We have accomplished this now for all ages including the embryo.

The results summarized here add significantly to our understanding of the origins and nature of vestibular responses and ultimately to our understanding of vestibular ontogeny. These represent significant steps toward our goal of evaluating the role of gravity in the ontogeny of gravity receptors. Moreover, these studies provide important insights that may lead to the successful application of the new vestibular test in the diagnosis of the dizzy human patient.

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Self-Generating Bending Moments in Root Gravitropism

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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 a new direction has emerged however. 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, 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.

Our project is yielding new information about the biomechanical parameters associated with root growth and development during seedling germination. This new information has developed on three levels. First, we have determined that horizontally oriented corn roots develop significant bending forces during the gravitropic response, generating up to 68 mN of load on a restraining platform which prevents them from bending. This represents a considerable ability to do mechanical work in moving soil particles or other obstacles, and demonstrates the critical role of the root's ability to determine the direction of the gravity vector and the importance of mechanical factors in the successful establishment of the seedling. Second, we have investigated the effect of tip load on vertically oriented, growing, corn roots. In these studies we investigated the relationship between the rate of root growth, (linear rate of root extension) and the magnitude of an axially applied restraining force. We have found an unexpected but clear cut independence of rate of growth on tip load, with growth rates remaining essentially constant from 0 mN tip load to 100 mN tip load. These results again relate to the ability of emerging seedling roots to perform mechanical work during the critical phase of germination when the root is attempting to penetrate the soil. Third, we have developed a new method for the determination of cell and tissue turgor pressures, using a simple, repeatable, and non-destructive method to obtain rapid information about internal cell pressures. This method again relates to our ability to obtain basic biomechanical information from growing plant systems.

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.

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Gravitropic Signal Transduction in the lazy-2 Tomato Mutant

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-40-00-00

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Expiration: 6/96

FY 1995 Funding: $51,163

Students Funded Under Research: 0

Task Description:

The proposed 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 (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 suppression of that mutation. During the current granting 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 manipulation can compensate for the absence of gravity in regulating stem development and orientation.

No additional data was provided by the investigator for this research.

Molecular Genetics of Root Thigmoresponsiveness in Arabidopsis thaliana

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Funding:

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Expiration: 3/97

FY 1995 Funding: $119,665

Students Funded Under Research: 4

Task Description:

The direction of root growth is dictated by a variety of environmental factors. These include the direction of the gravity vector (gravitropism), the direction of light (phototropism), gradients in water (hydrotropism), temperature (thermotropism) and ion channels (chemotropism). At any time, roots will decide on the direction of their growth by integrating the information provided by such environmental stimuli. The efficiency of this process will condition the levels and quality of plant productions. Unfortunately, while growing towards better microenvironments, roots will also encounter physical obstacles. They will have to detect such obstacles and respond to their presence by reorienting their growth to avoid them. The general objectives of this proposal are to understand the molecular mechanisms associated with touch sensing and response in plant roots. In the long term, these data should help us to understand how various environmental cues (including gravity and touch) interact to define the general direction of root growth on earth as well as under the microgravity environment of space.

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 protein will also be immunolocalized in plant organs, tissues, cells and subcellular compartments. 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.

My laboratory is specifically interested in defining the molecular mechanisms by which plant roots use gravity and touch stimuli to direct their growth. The specific objectives of this project are to define the processes involved in touch sensing and response by *Arabidopsis thaliana* roots, using molecular genetic strategies.

As a preliminary step toward that objective, we have defined the pattern of root growth for wild type seedlings of various ecotypes growing on agar surfaces. We have shown that wild type *Arabidopsis* roots from some ecotypes (e.g. Col) grow vertically downward on such vertical surfaces. However, wild type roots from other ecotypes (e.g. Ler, WS, C24) grow downward away from the vertical, to their right, tending to reach a specific vector at some angle from the vertical. We have identified three semi-dominant mutations which exaggerate that right slanting root growth phenotype. We have further shown that the right slanting root growth phenotype is not dependent upon gravitropism or phototropism; it is not a tropic response to any environmental vector or gradient, and it is purely surface-conditional. It probably derives from a surface-conditional circumnutation process.

To identify mutants affected in the ability of their roots to sense and respond to a mechanical stimulus, we have used the wavy root growth assay developed by Okada and Shimura (1990). When *Arabidopsis thaliana* seedlings grow on the surface of a medium containing high agar concentrations and tilted 30° backward, their roots develop a wavy pattern of growth which is accompanied by a reversible rotation of the tip at the elongation zone. Okada and Shimura (1990) postulated that the wavy pattern of root growth derives from a combination of gravitropism and thigmotropism, but Simmons et al. (1995) argued that it derives essentially from a combination of gravitropism and circumnutation. Using a 3-dimensional analysis of the wavy pattern of root growth, we have shown that the Simmons et al. model is oversimplified, and that root gravitropism probably derives from a combination of gravitropism, thigmotropism and/or other surface-derived responses.

To better define the mechanisms involved in root waving, we have identified a number of mutants affected in that process. We found that all agravitropic mutants develop an altered -often loopy- wavy pattern of root growth on tilted agar surfaces, confirming the involvement of gravitropism in that response. Additionally, we have identified 7 mutants developing dampened waves under these conditions, 3 mutants developing loose waves, 2 mutants developing loopy roots and 15 mutants developing compressed waves. We have cloned and characterized one gene defined by mutations conferring a compressed pattern of root waving on tilted agar surfaces (WVC1). We have shown wvc1 corresponds to a mutation in the previously characterized ASA1 gene, which codes for the anthranilate synthase enzyme. That enzyme is involved in the biosynthesis of various compounds including tryptophan and auxin (Niyogi and Fink, 1992). Interestingly, its expression is strongly induced by wounding and by compatible plant-pathogen interactions (Niyogi and Fink, 1992). These data strongly suggest that auxin and/or other products of that biosynthetic pathway are important regulators of root waving in *Arabidopsis thaliana*. Work is in progress to determine if ASA1 expression is touch-inducible.

Ca^{2+} is probably a second messenger in the transduction of mechanical signals in plant cells (Knight et al., 1991). Therefore, we have introduced the APOAEQUORIN gene into *Arabidopsis thaliana*. APOAEQUORIN codes for a protein which, in the presence of coelenterazine, emits light in a cytosolic Ca^{2+}-dependent fashion (Knight et al., 1991). Using that *in vivo* Ca^{2+} reporter system, we have shown that touch induces a transient rise in cytosolic Ca^{2+} levels in stimulated root or shoot cells, as expected (Sedbrook et al., 1996). Therefore, that system will allow us to test the possibility that some wavy mutants are affected in their ability to respond to a touch stimulus by a transient rise in cytosolic Ca^{2+} levels.

To better define potential sources of variation in the levels of cytosolic Ca^{2+} in our touch induction assays, we have investigated the effect of anoxia and circadian rhythms on the cytosolic Ca^{2+} levels in *Arabidopsis thaliana*. The data showed that *Arabidopsis thaliana* seedlings respond to anoxia by a transient, biphasic and organ-specific rise in cytoplasmic Ca^{2+} levels (Sedbrook et al., 1996). We have also collaborated with Carl Johnson, Takao Kondo, Marc Knight and Tony Trewavas to show that the cytosolic and chloroplastic Ca^{2+} levels follow a circadian rhythm in *Arabidopsis thaliana* and *Nicotiana tobacum* (Johnson et al., 1995). These important results will allow us to eliminate potential artifacts related to coelenterazine pretreatment (hypoxia) and to circadian rhythms in our assays.

In conclusion, our research over the last year has allowed us to define the patterns of root growth for wild type *Arabidopsis thaliana* seedlings on vertical agar surfaces. It has allowed us to identify a number of mutants affected in the ability of their roots to respond to mechanical and/or surface-derived cues in their environment and direct their growth accordingly. The molecular analysis of one of these genes has allowed us to postulate that auxin may be an important regulator of the wavy pattern of root growth on tilted agar surfaces. Finally, we have been able to optimize the APOAEQUORIN-based Ca^{2+} reporter system to follow the touch-induced pulses in cytosolic Ca^{2+} levels in *Arabidopsis thaliana*. This combination of genetic and molecular biology strategies will allow us to better understand the molecular mechanisms involved in the response of *Arabidopsis thaliana* roots to mechanical perturbations.

In soil, roots have to grow toward microenvironments which are optimal for growth and function. For instance, they have to find soil environments which provide a good source of mineral ions and water, as well as a good anchorage for the plant. To do so, they have developed the ability to use a number of environmental cues, including gravity, light, gradients in water, ions, chemicals and temperature, to direct their growth. However, even if they integrate that information and use it to grow toward optimal soil environments, they necessarily encounter obstacles in their path (soil particles, rocks, etc.). Therefore, they have developed a signal transduction system which allows them to sense such an obstacle and use that information to modify the vector of growth, thereby avoiding the obstacle. Clearly, this system conditions the level of plant productions by allowing roots to grow toward optimal soil environments independently of whether obstacles are found in their path.

Because the vector of root growth is determined by an integrated response to a number of environmental cues, one has to understand the involvement of each one of these cues in the final, integrated response of the plant. One also has to understand the interactions between the responses to several environmental cues if one wants to eventually be able to direct the process more carefully. This understanding will be crucial to optimize systems aimed at directing the patterns of root growth in microgravity environments where one essential player, gravity, is missing. The long term objective of our research is aimed at understanding the mechanisms by which roots sense and respond to mechanical perturbations, and how this response is affected by other environmental cues, such as gravity.

Publications, Presentations, and Other Accomplishments:

Hilson, P., Sedbrook, J., Caspar, T., Maher, P., and Masson, P. "Progress towards cloning the AGR locus (Abstract)." 6th International Conference on *Arabidopsis* Research, Madison, WI, June 7-11, 1995.

Hilson, P., Sedbrook, J., Caspar, T., Maher, P., and Masson, P. "Progress toward the molecular charcterization of two root agravitropic mutants (agr and arg1) in *Arabidopsis thaliana* (Abstract)." 11th ASGSB Annual Meeting, Washington, DC, October 25-29, 1995.

Hilson, P., Sedbrook, J., Caspar, T., Maher, P. and Masson, P. "Progress toward the molecular characterization of two root agravitropic mutants (agr and arg1) in *Arabidopsis thaliana*." 11th ASGSB Annual Meeting, Washington, DC, October 25-29, 1995.

Johnson, C.H., Knight, M.R., Kondo, T., Masson, P., Sedbrook, J., Haley, A., and Trewavas, A. "Circadian oscillations of cytosolic and chloroplastic free calcium in transgenic luminous plants." Science, vol. 269, 1863-1865 (1995).

Masson P. "Root gravitropism." BioEssays, vol. 17, 119-127 (1995).

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Masson, P., Rutherford, R., and Gallois, P. "A mutant of *Arabidopsis thaliana* shows an altered pattern of root waving when growing on a tilted agar surface." 11th ASGSB Annual Meeting, Washington, DC, October 25-29, 1995.

Rutherford, R., and Masson, P. "sku mutants of *Arabidopsis thaliana* show surface-dependent alteration of root growth direction." 6th International Conference on Abrabidopsis Research, Madison, WI, June 7-11, 1995.

Rutherford, R., and Masson, P. "sku mutants of *Arabidopsis thaliana* show surface dependent alteration of root growth direction (Abstract)." ASGSB Bulletin, vol. 9, no. 1, 37 (1995).

Rutherford, R., and Masson, P. "sku mutants of *Arabidopsis thaliana* show surface-dependent alteration of root growth direction." 11th Annual ASGSB Annual Meeting, Washington, DC, October 25-29, 1995.

Rutherford, R., Gallois, P., and Masson, P. "A mutant of *Arabidopsis thaliana* shows an altered pattern of root waving when growing on a tilted agar surface (Abstract)." 6th International Conference on *Arabidopsis* Research, Madison, WI, June 7-11, 1995.

Sedbrook, J., Kronebusch, P., Trewavas, A., and Masson, P. "Transgenic AEQUORIN reveals organ-specific cytosolic Ca^{2}+ responses to anoxia in *Arabidopsis thaliana* seedlings." 6th International Conference on Arabidopsis Research, Madison, WI, June 7-11, 1995.

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Are G Proteins Mechanosensors for Endothelial Cells?

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Funding:

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Students Funded Under Research: 1

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) phenotype and may influence the endothelial response to injury. Although not identical to gravitation, how EC 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 perturbation on EC 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 $G_{ia1,2}$, that corresponds temporally to the activation of the adenylate cyclase signal transduction pathway. The specific hypothesis to be tested in the proposed studies is that strain-induced loss of $G_{ia1,2}$ is caused by post-translational modification of this protein directed at at the carboxyl terminus. This hypothesis is based on preliminary data that show strain-induced loss of $G_{ia1,2}$ is limited to antisera that recognize the carboxyl terminus of $G_{ia1,2}$. The carboxyl terminus of $G_{ia1,2}$ contains a CAAX motif that is a well recognized site of post-translational modification such as prenylation.

Vitamin K1, an inhibitor of mono(ADP-ribosyl) transferase, prevents strain-induced activation of cyclic AMP dependent protein kinase (PKA) in bovine aortic endothelial cells. Previous data obtained in our laboratory demonstrate that cyclic strain causes a transient loss in the immunoreactivity of the inhibitory G protein ($G_{ia1,2}$) in endothelial cells that corresponds temporally to an elevation of adenylate cyclase activity, cyclic AMP accumulation and protein kinase A activity. It is our hypothesis that the loss of $G_{ia1,2}$ immunoreactivity is caused by post-translational modification of the protein directed at the carboxyl terminus. This hypothesis is based on our observations that strain-induced loss of $G_{ia1,2}$ immunoreactivity is limited to antisera that recognize the carboxyl terminus of $G_{ia1,2}$ and not to antibodies that recognize different regions of $G_{ia1,2}$. This hypothesis is supported by our recent finding that preexposure of endothelial cells to Vitamin K1 (10 (M) for 24 hours prevented strain-induced activation of protein kinase A activity. Since Vitamin K1 is an inhibitor of mono ADP-ribosylation of proteins in eukaryotic cells, these data suggest that strain-induced ADP-ribosylation of Gia1,2 may be the post-translational modification responsible for the loss of $G_{ia1,2}$ immunoreactivity.

Further support for the hypothesis that strain causes ADP-ribosylation of $G_{ia1,2}$ was obtained by the next study.

Pertussis toxin catalyzed ADP-ribosylation of the inhibitory G protein ($G_{ia1,2}$) in endothelial cell lysates is blocked by pre-exposure of cells to cyclic strain. In order to demonstrate strain-induced ADP-ribosylation of $G_{ia1,2}$, we measured the ability of pertussis toxin to ADP-ribosylate $G_{ia1,2}$ in endothelial cell lysates obtained after exposing cells to cyclic strain. We wished to determine whether cyclic strain-induced ADP ribosylation of $G_{ia1,2}$ would prevent subsequent ADP-ribosylation by pertussis toxin. It is well known that *in vivo* treatment of cells with pertussis toxin will prevent subsequent *in vitro* ADP-ribosylation and is in fact used to confirm *in vivo* efficacy of pertussis toxin treatment. Preexposure of endothelial cells to cyclic strain for 15, 30 and 60 minutes, led to a time-dependent loss of ADP-ribosylation by pertussis toxin. The data represent the densitometric summary of 3 experiments conducted under identical conditions. Studies to be conducted in the next funding period will include the following: 1) to determine whether strain-induced loss of $G_{ia1,2}$ immunoreactivity is blocked by Vitamin K1, 2) to determine whether other inhibitors of ADP-ribosylation such as nicotinamide can also prevent strain-induced loss of $G_{ia1,2}$ immunoreactivity and activation of protein kinase A and 3) to determine whether endothelial cells labeled with NAD substrate will exhibit direct ADP-ribosylation of immunoprecipitated $G_{ia1,2}$.

Compactin, an inhibitor of isoprenylation, does not prevent strain-induced activation of cyclic AMP dependent protein kinase (PKA) in bovine aortic endothelial cells. Contrary to preliminary data obtained earlier, subsequent experiments with compactin to block isoprenylation failed to show prevention of strain-induced activation of protein kinase A activity in bovine aortic endothelial cells. Pre-exposure to compactin (10 (M) for 24 hours did not affect strain-induced activation of protein kinase A activity (Mean+SE; (n=4) Control, 19.1+4.1; Compactin 22.3+5.0; Strain 28.0+7.1; Strain + Compactin, 29.2+8.2). Prior to ruling out any effect of isoprenylation, we will examine the effects of other HMG-CoA inhibitors such as pravastatin, lovastatin and simvastatin. If these agents are effective in preventing strain-induced loss of $G_{ia1,2}$ immunoreactivity and activation of adenylate cyclase activity, cAMP and PKA, then we will pursue studies to investigate whether cyclic strain can cause isoprenylation of $G_{ia1,2}$.

The objective of these studies is to examine the effect of mechanical perturbation on endothelial 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. Future studies with altered gravitational states as well as flight studies will be required to confirm this hypothesis.

Publications, Presentations, and Other Accomplishments:

Du, W., I.Mills and B.E. Sumpio "Cyclic strain causes heterogeneous induction of transcription factors, AP-1, CRE binding protein and NF-kB, in endothelial cells: species and vascular bed divesity." J. Biomechanics, (in press).

Mills, I., K.Kamal and B.E.Sumpio. "Cyclic strain causes ADP-ribosylation of the inhibitory G protein (Gia) in endothelial cells (EC)." FASEB J., (in press).

The Role of Actin Cytoskeleton in Auxin Transport and Gravitropism

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Funding:

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Initial Funding Date: 4/95

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FY 1995 Funding: $79,994

Students Funded Under Research: 4

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 still remain unclear. The polarity and quantity of auxin transport are controlled at the site of auxin transport that act at the site of auxin efflux. The critical first steps in the dissection of the regulatory pathway of auxin transport are characterization of this inhibitor binding protein and determination of the mechanisms by which this regulatory protein controls auxin efflux from plant cells. Recent evidence from this laboratory indicates that the regulatory or inhibitor binding subunit of the auxin efflux carrier from zucchini is associated with the actin cytoskeleton. In recent years, the role of the actin cytoskeleton has been shown to be more than structural. This filamentous network functions in intracellular movement, polarity development, and integration of cellular signals in a variety of organisms. Although the role of the actin cytoskeleton in the growth and development of higher plants has so far received limited study, the cytoskeleton has been suggested to be important in both perceiving and responding to environmental signals such as gravity. The plant cytoskeleton is uniquely suited to act as the signal transducer from the membrane and extracellular matrix, which sense external events, to the auxin transport stream which allows response to these signals.

The experiments in this proposal are designed to test the hypothesis that polar auxin transport is regulated by interactions of the regulatory protein of the auxin efflux carrier with the cytoskeleton. In order to understand the nature of the regulation of auxin transport, the interaction between the inhibitor or napthylphthalamic acid (NPA) binding protein of the auxin efflux carrier and actin must be further delineated. A basic understanding of this interaction will allow the development of purification approaches for isolation of the NPA binding protein. The purified NPA binding protein will facilitate preparation of molecular probes which can be used to further explore the regulation of this protein. Using these molecular probes and basic biochemical approaches, the interactions between the NPA binding protein and the actin cytoskeleton will be examined and changes in this association during gravity response will be analyzed. These experiments are designed to provide insight into the mechanisms by which polar auxin transport is controlled and by which this regulation of transport is modulated during gravitropism.

The goal of this research is to examine the actin association of an auxin transport protein and to determine the role of this cytoskeletal association in plant gravitropism. This year, a graduate student

supported by NASA completed a study designed to demonstrate that the NPA binding protein (NBP) of the auxin efflux carrier is specifically associated with actin. His completed thesis, which is now being condensed for publication, uses several approaches which all suggest that this protein interacts with the actin cytoskeleton and not microtubules. The approaches used to demonstrate this interaction included sucrose density gradients to separate cytoskeletal polymers from solubilized membrane components and addition of reagents that specifically interact with and stabilize either the polymeric or monomeric forms of actin and microtubules to alter the cytoskeletal association of the NBP. We have also applied these procedures to release the NBP from purified plasma membranes, toward the goal of purification of the NBP. We have preliminary data which suggests that the activity of the NBP is dependent upon cytoskeletal association. As a result, it will be difficult to follow this protein through a purification procedure using an assay that depends upon activity of the protein. We have established collaborations with two other researchers to develop approaches to covalently tag this protein, so that it can be followed through purification procedures by analysis of the covalent tag. Another graduate student, with partial NASA support, has almost completed a study which suggests that the cytoskeletal association of the NBP is reduced under conditions where auxin transport is reduced. We have developed procedures to quantify auxin movements in roots of both higher plants and algae and have begun to ask if alterations in cytoskeletal dynamics will alter the ability of these plants to transport auxin and respond to gravity. We have also obtained the microscopy facilities and expertise in order to visualize the actin cytoskeleton and to examine the effects of gravity on cytoskeletal organization. Our goal is to examine these events in the rhizoids of the algae, *Chara*, but we have encountered significant difficulty in obtaining preparations of soil free rhizoids. Collaborations with researchers at other universities have allowed us to make progress in this area.

The goal of this research is to examine the actin association of an auxin transport protein and to determine the role of this cytoskeletal association in plant gravitropism. This year, a graduate student supported by NASA, completed a study designed to demonstrate that the NPA binding protein (NBP) of the auxin efflux carrier is specifically associated with actin. His completed thesis, which is now being condensed for publication, uses several approaches which all suggest that this protein interacts with the actin cytoskeleton and not microtubules. The approaches used to demonstrate this interaction included sucrose density gradients to separate cytoskeletal polymers from solubilized membrane components and addition of reagents that specifically interact with and stabilize either the polymeric or monomeric forms of actin and microtubules to alter the cytoskeletal association of the NBP. We have also applied these procedures to release the NBP from purified plasma membranes, toward the goal of purification of the NBP. We have preliminary data which suggests that the activity of the NBP is dependent upon cytoskeletal association. As a result, it will be difficult to follow this protein through a purification procedure using an assay that depends upon activity of the protein. We have established collaborations with two other researchers to develop approaches to covalently tag this protein, so that it can be followed through purification procedures by analysis of the covalent tag. Another graduate student, with partial NASA support, has almost completed a study which suggests that the cytoskeletal association of the NBP is reduced under conditions where auxin transport is reduced. We have developed procedures to quantify auxin movements in roots of both higher plants and algae and have begun to ask if alterations in cytoskeletal dynamics will alter the ability of these plants to transport auxin and respond to gravity. We have also obtained the microscopy facilities and expertise in order to visualize the actin cytoskeleton and to examine the effects of gravity on cytoskeletal organization. Our goal is to examine these events in the rhizoids of the algae, *Chara*, but we have encountered significant difficulty in obtaining preparations of soil free rhizoids. Collaborations with researchers at other universities have allowed us to make progress in this area.

Publications, Presentations, and Other Accomplishments:

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Lomax, TL, GK Muday, and P Rubery "Plant Hormones: Physiology, Biochemistry, and Molecular Biology (Contribution: Auxin Transport)." Edited by: P.J. Davies Norwell: Kluwer Academic Press, pp 509-530, 1995.

Meday, G.K. "The Plant Cytoskeleton and Auxin Transport." Invited seminar at North Carolina State University, Horticulture Department, Raleigh, NC, October 9, 1995.

Muday, G.K. "NPA Binding Activity is Associated with the Cytoskeleton *in vitro*." At the International Plant Growth Substrances Association Meeting in Minneapolis, MN, July 14-18, 1995.

Muday, G.K. "Fucus as a Model System to Examine the Role of the Cytoskeleton in Auxin Transport." Invited Presentation at the Cytonet Retreat: The Plant Plasma Membrane, Cell Wall, Cytoskeletal Continum, Kona, Hawaii, May 12-17, 1995.

Muday, G.K. "NPA Binding Activity is Peripheral to the Plasma Membrane and is Associated with the Cytoskeleton." Keystone Symposia on the Frontiers in Plant Morphogenesis, Hilton Head, SC, March 29-April 4, 1995.

Muday, G.K. "Biochemical Dissection of an Auxin Transport Protein Suggests a Cytoskeletal Association." Invited Seminar, Bowman Gray Medical School, Biochemistry Department, January 17, 1995.

Muday, G.K. "Regulation of Auxin Transport by *in vitro* Changes in Cytoskeletal Association of the NPA Binding Protein." American Society of Gravitational and Space Biology Meeting, San Francisco, CA, October 19-22, 1994.

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Muday, GK and P Haworth "Tomato Root Growth, Gravitropism, and Lateral Development: Correlations with Auxin Transport." Plant Physiology and Biochemistry, vol 32, 193-203 (1994).

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Microgravity Effects on Early Reproductive Development in Plants

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Project Identification: 199-40-57-24
Solicitation: 01-13-94/GB

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FY 1995 Funding: $61,582
Students Funded Under Research: 4

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.

Completed work suggests that reproductive development and growth of *Arabidopsis* can be controlled by simultaneously lowering atmospheric levels of O_2 and CO_2. Atmospheres with less than 5% O2 and 100 ppm CO_2 adversely affect growth, metabolism, and reproductive development in *Arabidopsis*. This information is of use in interpretation of flight experiment results in closed plant growth chambers. Studies are currently underway to determine the effect of lowering the atmospheric O_2 level (CO_2 level held constant) on *Arabidopsis* growth, metabolism and reproductive development. Later this year, studies will be conducted to determine the effect of altering the atmospheric CO_2 level (O_2 levels determined from current studies) on *Arabidopsis* growth, metabolism and reproductive development, with special emphasis on CO_2 levels that are encountered in spacecraft environments and terrestrial elevated CO_2 scenarios.

In general, this work will increase our understanding of plant growth and development as it is affected by atmospheric composition. The low O_2 studies will provide baseline information that will allow

future researchers to grow plants, in space, exposed to oxygen levels lower than current earth levels, thereby decreasing O_2 requirements for a future space plant growth facility. The results of the altered CO_2 studies will help future researchers determine if plants can grow and reproduce in the elevated levels of CO_2 typical of a spacecraft environment. Because plants consume CO_2 (high concentrations of CO_2 are lethal for humans), plants could be used as a "CO_2 scrubber" in space environments. In terms of Earth-based benefits, the CO_2 studies will contribute information to the growing knowledge base related to the effects of the rising level of CO_2 in the Earth's atmosphere caused by anthropogenic and natural activities. The O_2 and CO_2 studies may also benefit controlled atmosphere-based industries in horticulture and ornamental floriculture.

Publications, Presentations, and Other Accomplishments:

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Skeletal Collagen Turnover by the Osteoblast

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Funding:

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FY 1995 Funding: $104,487

Students Funded Under Research: 2

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. In FY95, we have investigated the mechanisms of appearance of this gene further and found that cessation of mitosis does not appear to be associated with expression of collagenase but, rather, the actual mineralization process influences appearance of the enzyme.

We started work transfecting collagenase promoter constructs into osteoblastic cells. Since transfection of mineralizing cultures is almost impossible (requires dividing cells), we have tried different approaches. We have shown that expression of collagenase in rat osteoblastic cultures varies depending on the age of the animal with greatest basal expression observed in cells from 21 day old rats and about a 3-4-fold stimulation by PTH at all ages. Thus, to ascertain the differentiation element in the collagenase gene, we have used cultures of osteoblasts from rats of different ages, particularly 1 and 21 days old. Our experiments have shown greater promoter activity in the older animals but it is not completely absent in the cells from the 1 day old rats. Thus, we decided we needed to be completely sure of the culture conditions, differentiation state and transcription rate of the collagenase gene under these conditions before making any final conclusions regarding the promoter constructs.

Accordingly, we have carefully assessed the expression of differentiation markers in cells from 1 and 21 day old rats plated at different densities. This has revealed that the freshly isolated cells of 21 day old rats express more alkaline phosphatase than the freshly isolated cells from 1 day old rats. Subsequently, however, in both cases, the cultures de-differentiate and only redifferentiate slowly. The

cells from the older animals redifferentiate very slowly. We have concluded from these data that transfection must be conducted as soon as possible after culturing with fairly dense cultures.

In addition, we are determining the rates of transcription of the collagenase gene under various conditions, to be sure that transcriptional rate is the determinant of changes in mRNA abundance. When we have acquired this information we will return to the transfection experiments.

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 effected 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.

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Cook, T.F., Burke, J.S., Bergman, K.D., Quinn, C.O., Jeffrey, J.J. and Partridge, N.C "Cloning and regulation of rat tissue inhibitor of metalloproteinases-2 in osteoblastic cells." Arch. Biochem. Biophys., vol 311, 313-320 (1994).

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Pearman, A.T., Chou, W.Y., Bergman, K.D. and Partridge, N.C. " (Abstract) Parathyroid hormone activates the c-fos promoter through a cyclic AMP response element." Bone, Vol 16, supplement 1, abstract 485, (1995).

Pearman, A.T., Chou, W.Y., Bergman, K.D., Pulumati, M.R. and Partridge, N.C. "Parathyroid hormone stimulates the c-fos promoter through CREB phosphorylation and binding to the major CRE." J. Bone Min. Res., vol 10, supplement 1, S529, (1995).

Mechanotransduction and the Cortical Cytoskeleton: What is the relationship?

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Solicitation:

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FY 1995 Funding: $50,000
Students Funded Under Research: 1

Task Description:

The plasma membrane of epidermal cells contains a mechanosensitive calcium channel that is modulated or inhibited by several agents which are known to inhibit gravitropic reception with some specificity; it is therefore quite possible that this channel is a primary gravitropic force transducer in these cells. Further, consistent with the prevalent idea that the cytoskeleton is somehow associated with gravity reception, we have found five factors which modify channel action and also modify the cortical cytoskeleton. Because the relationship between channels and cytoskeleton is believed intimate, we propose that it should be possible to develop a long list of modifiers with parallel influences. Detailed comparison of many such twin effects should lay a foundation for modelling the macromolecular architecture of the gravity transduction system. Since the literature already provides a long list of factors (including all the major plant hormones) that control the arrangement of the cortical cytoskeleton, we propose to screen for possible effects of these on the mechanosensory channel. We anticipate that negative as well as positive results will be of value in developing the model, and that information about effects of hormones, herbicides, and other regulators on the channels will be of immediate, independent interest to a broad audience of plant biologists.

By the time the grant was actually funded, I had lost my patch clamper to a postdoctoral position which had current, as well as long-term, funding. At the same time, two intellectual developments in the lab suggested a more powerful approach to the problem defined in the original statement. These were discussed with the Program Chief, who agreed that an alteration of approach was desirable.

First, still-unpublished experiments by my former patch clamper and by a collaborator in Japan had by then shown the mode of action of some of the compounds we were interested in testing, and we realized that we could predict the influences of a large number of compounds we were interested in. Thus, we felt challenged to jump ahead and look at the cytoskeletal environment more directly.

Second, expansion of our work on the computational optical sectioning microscope (COSM) which was built by my colleague James G. McNally here at Washington University showed that we had unique capability for observing the cytoskeleton directly, in living cells, during mechanostimulation. In principle, we have a method at hand for locating the channels by this fluorescence microscopy as well. I was able to bring in a postdoctoral associate who was interested in pursuing this study. Also, because of the multiple facets of COSM work, and the immensity of each facet, we applied for a

NASA/FNET grant in sensory plant biology and received it. Therefore, several studies have gone on in parallel and the people supported by the latter have interacted usefully with the NASA postdoctoral fellow in learning new computer techniques, maintaining fragile equipment, and so on.

Though the coauthors of forthcoming papers from my lab were supported by different grants, the primary questions addressed by the postdoc supported by this research were: Are there cytoskeletal proteins (besides actin and microtubules) in plants homologous to those that make up the known group of major cytoskeletal players in animals? How are these distributed in the living cell? How is the distribution influenced by activity of the mechanosensory calcium channel we believe to be responsible for vectorial gravitropic stimulation and for the sensing of mechanical stimuli in general? Additionally, we hoped to visualize the channels with respect to cytoskeletal entities, but the first three questions proved to have such important answers and to require such intensive work to obtain them that we deferred this covisualization for future activities supported by the NASA/NSF collaborative grant.

We have identified a major here-to-fore unknown cytoskeletal structure in our representative experimental system, the onion epidermal cell and have named this structure the endomembrane sheath.

The endomembrane sheath appears to anchor at adhesion sites, to which we postulate the gravitropic sensor channels are also tethered. A paper on these adhesion sites has been accepted by the international journal Protoplasma, subject to some revisions that are nearly complete but not subject to a second examination of the paper by the reviewers. The contribution to this paper by this research (which funded a postdoc) was to extract and separate and immunologically identify the key adhesion protein integrin.

The adhesion sites are postulated to be of importance for the activation of the mechanosensory channels (see review article in Protoplasma 182:1-9), and they and the endomembrane sheath are presumed important for the internal signaling sequelae that follow activation.

We believe this study to be a breakthrough in understanding plant sensory biology as well as more general aspects of plant cell biology. The most immediate outcome we foresee is that it may give insight into how to achieve better crop resistance to stress. We are currently working in collaboration with three other labs on proteins known to protect plants from stress. We believe they may exert their macroscopic effect by regulating at the microscopic level how the endomembrane sheath responds to diverse forms of stress (including low temperature stress), and in so doing control both cellular architecture and cellular biochemistry (metabolism and protein synthesis). Also, preliminary success in covisualizing proteins that confer stress resistance suggests that a large agricultural gain may result from pursuit of the role of these cytoskeletal proteins. Finally, understanding how the mechanosensory channels, adhesion sites, and endomembrane sheath interact could predict a great deal of the plant's response to microgravity, thus saving costly "look-and-see" experiments in space vehicles.

Publications, Presentations, and Other Accomplishments:

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Calcium Messenger System in Gravitropic Response in Plants

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Funding:

Project Identification: 199-40-57-08

Solicitation:

Initial Funding Date: 7/95

Expiration: 6/96

FY 1995 Funding: $65,000

Students Funded Under Research: 8

Task Description:

The primary goal of our studies is to understand how plants detect the gravity signal. It is becoming clear that external signals elevate cytosolic calcium which activates calmodulin, a ubiquitous calcium binding regulatory protein that is known to mediate calcium action. Advances in the use of the photoprotein aequorin, have opened exciting possibilities in calcium research. This has enabled plant scientists to quantify signal-induced transient changes in free calcium in intact cells and tissues. Transgenic *Nicotiana* plants carrying the aequorin gene that can report changes in free calcium concentration during gravistimulation are being used. To accomplish this, the apoaequorin-coding region from the complementary DNA clone was fused to the CaMV 35S promoter and transferred to plants using the *Agrobacterium tumefaciens* binary vector system. Our ultimate goal is to establish a system to measure transient changes in free calcium concentration in intact seedlings under 1 g as well as under near zero g conditions and attempt to manipulate calcium levels to control the behavior of seedlings under microgravity conditions. The second aspect of our investigation involves studies on the role of calmodulin and calmodulin-binding proteins in gravitropism. We have cloned and characterized a plant calmodulin cDNA (PCM-1) that shows signal-induced changes in its expression. Our results suggest that transcriptional regulation of the calmodulin gene plays an important role in signal transduction. We are studying the effect of gravity on calmodulin gene expression by *in situ* hybridization and by studying the activity of the promotor fused to the ß-glucuronidase (GUS) reporter gene. Much of the diversity in response in plants is believed to be achieved by the modulation of the activity of the calcium and/or calcium-calmodulin-dependent protein kinases. Recently, we cloned and characterized a novel calcium/calmodulin-dependent protein kinase from plants. Six calmodulin genes from potato plants were also cloned and characterized. Among these genes, PCM-1 was unique because of its responsiveness to environmental signals. Sequence comparisons of different genes revealed that the deduced amino acid sequence of PCM-1 had several unique substitutions, especially in the fourth Ca^{2+}-binding area. Transgenic plants carrying sense or antisense construct of PCM-1 showed significant differences in growth and development. A novel kinesin-like gene with a calmodulin-binding region within the motor domain was recently cloned and characterized. The role of this gene in gravity signal transduction is being investigated.

Transgenic potato plants with altered growth and development: A transgene approach was taken to study the consequences of altered expression of the novel calmodulin isoform (PCM-1) on plant growth and development. Transgenic potato plants were produced carrying sense or antisense construct of

PCM-1 fused to a constitutive CaMV 35S promoter. Interestingly, these plants showed striking differences in growth and development. These findings on calmodulin have appeared in NASA Tech Briefs. The investigators involved in this study were pleased to receive certificates and checks from NASA for this work. Washington State University has applied for patent protection for the transgenic plants.

Cloning and characterization of a chimeric calcium/calmodulin-dependent protein kinase (CCaMK) gene with a neural visinin-like calcium-binding domain: CCaMK contains all eleven major conserved subdomains of the catalytic domain of serine/threonine kinases. Sequence comparisons revealed that CCaMK has high homology to Ca^{2+}/CaM-dependent protein kinases, especially in the kinase and CaM-binding domains (amino acid residues 1-338). The CaM-binding region of CCaMK (FNARRKLRAAAIASVL, residues 323-338) is similar to the CaM-binding domain (FNARRKLKGAILTTML, residues 293-309) of the subunit of mammalian CaMKII. The sequence downstream of the CaM-binding region of CCaMK (amino acid residues 339-520) does not have significant homology to known Ca^{2+}/CaM-dependent protein kinases. Further analysis of this region revealed the presence of three Ca^{2+}-binding EF-hand motifs that had the highest homology (52-54% similarity; 32-35% identity) to a family of genes belonging to visinin-like Ca^{2+} binding proteins which are found mainly in neural tissue.

A novel kinesin-like gene with a calmodulin-binding region within the motor domain: 35S labeled calmodulin was used to screen the expression libraries to isolate cDNAs encoding calmodulin-binding proteins. A kinesin-like gene (TCK1) that encodes a calmodulin-binding kinesin-like protein was obtained. The TCK1 cDNA encodes a protein with 1265 amino acid residues. Its structural features are very similar to those of known kinesin heavy chains and kinesin like proteins from plants and animals, with one distinct exception. Unlike other known kinesin-like genes from plants and animals, TCK1 contains a novel calmodulin-binding domain which distinguishes it from all other known kinesin genes. *E. coli*-expressed TCK1 binds calmodulin in a Ca^{2+}-dependent manner. In addition to the presence of a calmodulin-binding domain in the motor domain at the carboxyl-terminal, it also has a leucine zipper motif in the stalk region. The amino acid sequence at the carboxyl-terminal of TCK1 has striking homology with the mechanochemical motor domain of kinesins. The motor domain has ATPase activity that is stimulated by microtubules. Our results suggest that Ca^{2+}/calmodulin may play an important role in the function of this microtubule-associated motor protein and may be involved in the regulation of microtubule-based intracellular transport.

Calcium-dependent protein kinase genes in corn roots: Two cDNAs encoding Ca^{2+}-dependent protein kinase (CDPKs), CRPK1 and CRPK2 (corn root protein kinase 1 and 2) isolated from the root tip library of corn (*Zea mays L.*, cv Merit) and their nucleotide sequences were determined. Deduced amino acid sequences of both the clones have features characteristic of plant CDPKs, including all 11 conserved serine/threonine kinase subdomains, a junction domain and a calmodulin like domain with four Ca^{2+}-binding sites. Northern analysis revealed that CRPK1 mRNA is preferentially expressed in roots, especially in the root tip, whereas, the expression of CRPK2 mRNA was very low in all the tissues tested. In situ hybridization experiments revealed that CRPK1 mRNA is highly expressed in the root apex, as compared to other parts of the root. Partially purified CDPK from the root tip phosphorylates syntide-2, a common peptide substrate for plant CDPKs, and the phosphorylation was stimulated 7-fold by the addition of Ca^{2+}. Our results show that two CDPK isoforms are expressed in corn roots and they may be involved in the Ca^{2+}-dependent signal transduction process.

Plant organs respond to different environmental signals such as gravity and light. Roots show a positive response to gravity while stems respond negatively. A better understanding of the gravity sensing mechanism in plants would ultimately help in growing plants under microgravity conditions in space. The gravitropic response is separated into three phases - perception, transduction, and response. Calcium has been shown to regulate diverse physiological processes in plants. In recent years, it has become evident that calcium plays a unique role in all three phases of gravitropism. It is believed that calcium/calmodulin-dependent protein kinases are involved in amplifying and diversifying calcium-

mediated signals. A better understanding of the calcium-signaling pathway will help in understanding how plants perceive signals such as gravity. Furthermore, the information derived from these studies could be used to manipulate plant growth and development under microgravity conditions.

Publications, Presentations, and Other Accomplishments:

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Wang, W., Takezawa, D., Narasimhulu, S.B., Reddy, A.S.N. and Poovaiah, B.W. "A novel kinesin-like gene with a calmodulin-binding region within the motor domain." Plant Mol. Biol., (in press).

Mechanism of Auxin Action in Root Growth/Gravitropism

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Students Funded Under Research: 4

Task Description:

An important question in root gravitropism research is the identity of the substance (or substances) that initiates and drives the asymmetric cell elongation which ultimately causes root gravitropism. There is a substantial body of evidence which suggests auxin (IAA) may be the gravitropic effector in roots. However, there are also serious questions and inconsistencies which cast doubt on auxin-based models. I argue this controversy is unlikely to be resolved until we better understand the cellular and molecular events associated with the retardation of cell elongation caused by hormone levels of 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 described in this proposal represent steps which may help to rectify this situation and provide new tools to test whether IAA in indeed the gravitropic effector in roots.

I propose to 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, I 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 proposal 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 pride 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.

As a first step in attempting to isolate auxin up- and down-regulated genes in tomato seedling roots, we constructed cDNA libraries for auxin-depleted and auxin-treated root sections using the Lambda Uni-ZAP XR vector system. These libraries were then screened by the plus/minus method. Two attempts failed to isolate auxin-regulated clones. This indicates auxin may not alter the expression of abundantly expressed messages, at least during the early response time points we are examining. PCR-based subtractive hybridization is presently being employed to identify lower abundance up- and down-regulated messages. The extremely high sensitivity of this approach should allow us to isolate the

relatively rare transcripts typically central to mediation of early signal transduction responses. We have also made progress in testing the hypothesis that auxin causes the down-regulation of specific plasma membrane H^+-ATPase transcripts in roots. Tomato roots express three H^+-ATPase isoforms. Quantitative RT-PCR of these transcripts from total RNA followed by high stringency hybridization indicates transcripts of one of the isoforms are indeed down-regulated while transcripts of the other two isoforms are not altered. We are presently attempting to verify this exciting result using RNase protection assays.

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 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.

Publications, Presentations, and Other Accomplishments:

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Hyper-G Studies of Vestibular Maculas Neural Plasticity

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Project Identification: 199-40-12-01
Solicitation: 93-OLMSA-07
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FY 1995 Funding: $129,814
Students Funded Under Research: 0

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 maculas of rats exposed to altered gravity. The new investigation focuses 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. During the first year, morphological studies of maculas of rats centrifuged at 2-g for 14 days and for 4 days will be conducted, to compare findings with SLS-2 results and to examine adaptive effects at this early stage. This tissue is already embedded and a pilot study has been carried out. The results indicate that synapses of type II hair cells decline in hypergravity, an effect opposite to that observed in microgravity. Ground-based electrophysiological data will also be obtained during year 1 and, once complete, correlated anatomical and physiological studies will begin. Rats will be centrifuged for 4 days at 2-g initially, since the results will be relevant to planning space research on early adaptive changes. Rats chronically implanted with electrodes will be tested on a linear sled in populations exposed to hyper-g and during readaptation to 1-g will be recorded. Spontaneous and stimulated firing rates will be analyzed for rate, gain, coefficient of variation and functional polarization vector. A parallel group of rats will be used to correlate synaptic features with the discharge properties observed. Synapse type, size and distribution will be characterized using Recon software developed in the Biocomputation Center, and for analysis of variance using SuperANOVA TM software. Comparison of morphological results with those obtained from the previous 14 day and 4 day experiments will test experiment reproducibility. New understanding of macular dynamics, plasticity and behavioral responses should emerge from this correlated anatomical and physiological investigation. The information generated should be useful in planning future experiments on neural plasticity in altered gravity environments.

Experiments with thin film electrodes were begun using available electrodes designed by other investigators for multichannel recording from nerve fibers. These electrodes were not appropriate for recording from all eight channels within Scarpa's ganglion; the spacings between recording sites were spaced too far apart. However the unique "sharpened" tips of these electrodes made them the only electrodes capable of penetrating the neural sheath surrounding the VIIIth cranial nerve. By testing these

electrodes in a series of acute recording studies we were able to design a first generation of electrodes more suited to Scarpa's ganglion. We used this first generation design to determine accurately the stereotaxic coordinates and best angle of approach to Scarpa's ganglion, to tune the size and separation of recording sites, and to work out the overall logistics of placing and securing a chronic electrode. One of the first steps was to design a method for holding the electrode and head connector. The design and method developed here for holding thin film electrodes for stereotaxic implantation of a chronic electrode is a totally new one. The devise consists of a polycarbonate frame with a modified forceps angled at 24 degrees and a bushing to hold the head connector for attachment to the surface of the skull. When the electrode reaches proper depth in the brain, the head connector is suspended ~1 to 2 mm above the surface of the skull. This method has proved to be a very good one for our purposes. Methods of sterile surgery and use of gas anesthesia were studied and practiced in acute animals. Sham operations were performed to determine the method for placing the chronic electrodes, sterile surgical techniques and long term stability of the head connectors. It was also necessary to learn whether rats with implants can be housed together. To study this, four rats had head connectors without electrodes attached to their skulls. They were housed two to a cage in specially modified plastic cages. The design assured that the head connector would not be banged, scraped or tangled in the standard wire cage top. Results showed that there was no problem housing the rats in pairs as is common practice on the centrifuge.

In addition to developing electrodes and methods for their implantation, a histologic study of rat temporal bone was completed to determine the exact location and extent of Scarpa's ganglion. It was determined that the ganglion cells were accessible from within the calvarium. Measurements of rat skulls were made to determine variability in size among animals of similar age and to learn about changes that occur with age and weight. These data were used for correlation with the location of Scarpa's ganglion to determine potential variability of electrode placement.

Designs for the second generation of electrodes have been submitted to the University of Michigan where the electrodes are made. These new electrodes should be available soon for use in chronic studies of the responses of multiple vestibular afferents to linear accelerations, normal for Earth and encountered in altered gravity. Such work in the vestibular system, using multi-channeled electrodes, has not been attempted previously to our knowledge.

This research seeks to answer the fundamental question 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 work will have broad applications in 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, the work involves the use of newly developed multichannel electrodes. Results will greatly improve our knowledge of the activity in several different nerve fibers transmitting information resulting from a stimulus applied simultaneously to various parts of the receptor, each of which 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 and 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.

Publications, Presentations, and Other Accomplishments:

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Cellular Bases of Light-stimulated Gravitropism

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Funding:

Project Identification: 199-40-57-01

Solicitation:

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FY 1995 Funding: $65,000

Students Funded Under Research: 6

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 Ca2+ 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 [Ca^{2+}] in fern rhizoid cells.

A major project goal of this year was to identify a cDNA for a plant annexin and from the sequence of this cDNA deduce the primary amino acid sequence for that annexin. This goal was accomplished by an analysis of the monthly publication of thousands of new cDNA sequences for genes encoded in the genome of the plant *Arabidopsis*. Our analysis reveals that plant annexins preserve many of the key features of annexins previously described only in annexins isolated from animal sources. This breakthrough will allow for the design of genetic experiments that will more rigorously test the role of annexins in plant gravitropism. A second major accomplishment was to document that annexin expression in germinating fern spores was up-regulated by the same red light signal and photoreceptor (phytochrome) that modulates gravitropic growth in plants, and that the distribution of annexins in these spores exactly coincided with regions of active secretion of new wall materials and of new growth at the tip of rhizoids emerging from the spores. These findings correlating annexin distribution with new cell growth, which were published in *Planta*, indicate that annexins are important components of plant cell growth machinery and further encourage our investigation of whether annexins are involved

in mediating the new growth asymmetry characteristic of gravitropism in higher plants. A third major accomplishment was to develop a new single-cell model system for studying gravity responses in plants. We found that in single germinating spores of the fern *Ceratopteris richardii* the direction of migration of nuclei and the subsequent growth direction of rhizoids can be oriented by gravity. The germination of these spores can be initiated and synchronized by photoactivated phytochrome, and this allowed us to document that the time of graviresponsiveness of the spores was restricted to a period about 6 hours long that typically occurred between 5 and 10 hours after germination was initiated. Learning what changes in the spores when they first develop the capacity to respond to gravity should greatly enlighten the cellular basis for graviresponsiveness. This important advance was published in *Planta* early in FY95.

Of course a wide variety of new questions arise out of the above accomplishments. Here we will highlight only a few of these questions. Answering these questions will be the goal of future work on the project. Our first accomplishment raises the question of how many different annexins there are in plants (there are at least 11 different ones in animals), how different annexins are distinguished from one another (the distinctive sequences in animals are typically at the N-terminal region), and how different annexins differ from each other functionally. Our second accomplishment raises the question of whether the annexin-growth connection found in germinating fern spores will hold true also in higher plants. Our third accomplishment raises the question of whether the development change that allows gravity to fix the polarity of nuclear migration in ferns involves new gene expression or is controlled post-transcriptionally.

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 man 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 that can affect man on Earth and in space. 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 the common man in inextricably linked to his 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 FY95 do contribute to that improved understanding, and thus indirectly benefit common man.

Publications, Presentations, and Other Accomplishments:

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Hsieh, H.-L., C.-G.Tong, C. Thomas, and S.J. Roux "(Abstract) Light-modulated abundance of the mRNA for a calmodulin-regulated, chromatin-bound NTPase in pea." Plant Physiology, vol 108 (Supplement), 99 (1995).

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Osteoblastic Response to Gravity in Transgenic Mice

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Funding:

Project Identification: 199-40-27-14

Solicitation:

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Expiration: 3/96

FY 1995 Funding: $60,000

Students Funded Under Research: 0

Task Description:

No additional data was provided by the investigator for this research.

Re-Evaluation of the Role of Starch in Gravitropic Sensing

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Funding:

Project Identification: 199-40-57-28

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Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $111,857

Students Funded Under Research: 5

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* (N s458) 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. However, these kinetics must be redetermined since it was recently established that that Arabidopsis roots are negatively phototropic. Preliminary data show that starch-deficient roots are more strongly phototropic than WT roots. Since the light was overhead in previous experiments, existing data overestimate gravitropic sensitivity, especially for starch-deficient roots. Furthermore, it now appears possible that TC7 and an isolated line, MG421, are both double mutants containing genetically separable, although closely linked, loci for starch- and for gravi-. Thus, to test whether starch is essential for full sensitivity, it will be necessary to measure gravitropic sensitivity in starch-deficient lines of *Arabidopsis* that are not influenced by root phototropism or by a separate gravitropism locus.

With respect to root phototropism, we propose to determine the extent to which unilateral illumination influences the measurement of gravitropic sensitivity by comparing the effects of light position on plants rotating on a turntable and also by shutting off overhead lights during brief periods of gravistimulation (horizontal placement). These methods will also be used to determine whether the presence of either of two mutant photo- (aphototropic roots) mutations (rpt 1 and 2) influences gravitropism. To eliminate the influence of phototropism, both rpt mutations will be separately introgressed into all lines whose gravitropic sensitivity will be tested (Columbia and Estland wild-types, TC75, TL255 and segregants from MG421). If a significant portion of the apparent gravitropism in TC7 (or TC75) can be attributed to negative phototropism, then this would strengthen the starch-statolith hypothesis.

In order to determine whether, in fact, both MG421 and TC75 are truly double mutants, (1) more genetic data will be gathered from "dihybrid" crosses to isolate segregants (recombinants), and (2) both phenotypes will be mapped using multiple morphological marker lines. If starch- gravi+ (the "+" refers to gross phenotype, not threshold sensitivity) lines are isolated, they will then be used for more critical studies of whether the absence of starch depresses gravitropic sensitivity. The aphototropic rpt

gene(s) will be bred into these lines to eliminate a second confounding factor on the measurement of gravitropism. If the starch-gravi+ (single) mutant is actually impaired in gravitropism, then this would still support the hypothesis that the mass of starch participates in sensing. If, however, this mutant was starchless but fully competent gravitropically, then this would reverse previous conclusions about the importance of starch and effectively eliminate the starch-statolith hypothesis. Other starch-deficient mutants (TL255 from *Arabidopsis* and N S458 from *Nicotiana*) will be analyzed genetically to eliminate the unlikely possibility that they are also double mutants. Regardless of whether they have or lack a second mutation in gravitropism, they will provide important comparative data for the effects of starch-deficiency from a different locus (TL255) or a different genus (N S458).

One of the major questions we are examining is the interaction of light and gravitropic sensing. We have been using several starchless or starch-deficient mutants in *Arabidopsis* and *Nicotiana* to test the hypothesis that it is the mass of the starch that provides the signal that tells the plant which way is up. As is well known, light interacts with gravitropism in many ways. In our grant, we are studying two of those interactions that affect sensing, directly or indirectly. One line of research is to make clear how much negative phototropism in roots *Arabidopsis* interferes with measurement of gravitropism, especially at threshold levels. A second line relates to our previous finding that hypocotyls of *dark-grown* starch-deficient mutants of *Nicotiana sylvestris* are severely disoriented with respect to gravity. We subsequently tested whether *light-grown* hypocotyls were as agravitropic. Plants were grown in the light to a seedling stage with lights overhead so that stems point upward towards the light. Seedlings were then reoriented to the horizontal in the dark. Surprisingly, gravitropism in light-grown plants is much closer to that of the wild-type (full component of starch) compared to dark-grown plants. Microscopy of light-grown plants shows that the starch levels in the mutant are essentially comparable to the wild-type suggesting that light is not acting by increasing the mass of the presumed gravisensor. Currently, the gravitropism of light-grown plants is being evaluated quantitatively at threshold doses to determine whether gravitropism in the mutants is depressed, if at all.

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 the common man or in new technologies. It does, however, address basic biological questions of widespread interest, i.e. 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 that is underway 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.

Publications, Presentations, and Other Accomplishments:

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Effects of Hindlimb Suspension on Skeletal Muscle Growth

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Funding:

Project Identification: 199-40-27-19

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Expiration: 3/97

FY 1995 Funding: $79,753

Students Funded Under Research: 7

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 3 days after HS when all mitotic divisions are abolished in the soleus muscle. After a complete cessation of divisions that lasts through 5 days, a low level of divisions resumes. Proliferations appear to remain suppressed for as long as the limb is non-WB 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 proposal 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.

Our studies suggest that extended periods of weightlessness (NWB) could have profound detrimental effects on growing skeletal muscles. Growing muscles placed in a nonweightbearing environment exhibit a rapid cessation of growth, as measured by the mitotic activity of satellite cells, which are responsible for the addition of myonuclei to the enlarging myofibers. Thus, periods of NWB lead to a net deficit in the number of myonuclei in a myofiber satellite cell mitotic activity in the growth-retarded muscle. However, our results thus far suggest that the increase is not sufficient to compensate for the net reduction in myonuclei sustained during the experimental period. These results suggest that growing muscle, subjected to prolonged periods of NWB could be permanently growth retarded, even if WB is reinitiated. The same situation appears to be the case in injured muscle. After injury, the early phase of muscle regeneration, characterized by the removal of necrotic myofibers, activation of satellite cells, and the formation of new myofibers, does not appear to be influenced by the weightless environment. However, the newly formed myofibers are influenced by the weightless environment in the same way as the growing fibers and their development (growth) is slowed.

Our investigation into the satellite cell population of growing skeletal muscles suggests it is composed of at least two subsets, which we have called the producer and reserve population. The producer population is intimately involved in the production of myonuclei. We hypothesize that this population is depleted during the early phase of the weightless period, and the delay in responding to WB is in part related to replenishing this population. Future studies will be directed toward obtaining evidence to support or refute this hypothesis.

We have begun investigation of the role of several muscle-specific transcriptional-regulatory factors in skeletal muscle during growth in WB and non-WB animals. Initial reports suggested that these factors (myogenin, MyoD, Myf5, MEF2C, Id) could have roles in the stability of the differentiated state in skeletal muscles. Because of the profound changes that the weightless environment has on skeletal muscle, we are investigating how expression of these myogenic factors is altered as a means to better understand the underlying mechanisms affecting these changes. Using *in situ* hybridization we have found that there is an apparent decrease in the expression of some but not all of these factors in non-WB muscles. The change in expression appears to be related solely to satellite cells. We have been unable to demonstrate any change in expression of these factors using Northern analysis after periods of 14 days of non-WB. Western analysis has shown phenotypic changes in myosin isoform expression in the non-WB muscles occurs between 14 and 28 days. We are currently analyzing these periods.

In summary, growing muscles exhibit rapid and profound response to NWB. The response to WB is not as dramatic, and as a consequence, the recovery process is long-term. The results of our studies to date strongly suggest that the recovery process may not be complete and may possibly lead to permanent growth deficits. Future work will investigate more completely the recovery process of immature muscles after acute and prolonged periods of NWB.

We are just now beginning to obtain a better understanding of the effects of weightlessness on growing skeletal muscle. The underlying mechanisms of growth that are influenced have yet to be determined. We have accumulated a great deal of evidence that suggests the myofiber-satellite cell unit is altered in a significant way and suspect that a particular compartment of satellite cells is most influenced by the weightless environment. DNA accumulation to the adult complement occurs over a relatively short period, and weightlessness suppresses the rate of DNA accretion of myofibers. As a result, myofibers may be permanently altered because once the growth period is completed, the ability of the myofibers to increase the rate of myonuclear accretion is diminished. The work being carried out will hopefully provide a means to modulate the growth process. A better understanding of the growth process will eventually afford the ability to modulate muscle growth 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.

Publications, Presentations, and Other Accomplishments:

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Space Biology Research Project

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Funding:

Project Identification: 199-99-17-03

Solicitation:

Initial Funding Date: 12/94

Expiration: 11/00

FY 1995 Funding: $185,000

Students Funded Under Research: 5

Task Description:

The NASA Space Biology Research Associate Program has provided the opportunity to train scientists to conduct biological research in outer 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.

Since June 1, 1980, 109 Research Associate Awards have been made. The scientists who have completed this program have accepted positions in colleges and universities, with research laboratories, and with NASA. There have been over 206 publications in refereed journals and as many abstracts of papers presented at national and international meetings. By any measure, this is an excellent record of research achievements. In 1995, a three-month rotation at NASA Ames Research Center was made a requirement of the new Research Associates. One Associate has already completed half this requirements, and others are scheduled to follow.

Publications, Presentations, and Other Accomplishments:

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Growth Factors and Tension-Induced Skeletal Muscle Growth

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Funding:

Project Identification: 199-40-47-03
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1995 Funding: $218,281
Students Funded Under Research: 7

Task Description:

Three-dimensional mammalian skeletal muscle organs ("organoids") will be generated in tissue culture with computer-controlled mechanical cell stimulators. They will be used to study at the cellular and molecular level, tension/gravity-related skeletal muscle growth. 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 first, studying the growth of organoids implanted into syngeneic hosts, and second, studying the reversal of hindlimb suspension-induced atrophy in animals implanted with genetically modified organoids secreting recombinant growth hormone. The long term goals of this project are to establish mammalian muscle organoids as an appropriate system to study exercise attenuation of tension/microgravity-induced skeletal muscle atrophy in tissue culture, *in vivo*, and in space. The 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 muscle organoid 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") from either primary murine skeletal myoblasts or a murine myoblast cell line (C2C12) stably transduced with a gene for recombinant human growth hormone. A simplified growth chamber was designed with the gross geometry of a skeletal muscle whereby the mononucleated muscle cells could be cast in an extracellular matrix gel. Mechanical tension placed on the matrix embedded cells during their fusion and differentiation oriented the multinucleated myofibers longitudinally from end to end in the organoids. An enriched medium containing numerous growth factors was developed for the long term maintenance (3 to 4 weeks) of these mammalian organoids. Task 1 of the project was therefore accomplished during the first year of the project. These mammalian organoids will now be utilized for cellular and molecular level studies on tension/gravity regulation of muscle growth. Their long term survival in the modified bioreactor cartridges of the Shuttle's Space Tissue Loss Module will also be examined in the project's second year to assess their potential for future small payload flight

experiments of short duration. Longer duration studies with the mammalian organoids will also be possible in the Cell Culture Unit under development for the International Space Station Alpha.

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 exits for the use of the techniques developed as part of this project to tissue engineer human skeletal muscle organoids 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 organoids 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 of such tissue engineered muscle gene therapy techniques will be tested in animal models during the second year of the project.

Publications, Presentations, and Other Accomplishments:

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Gravity-Induced Polarity of Transport in Plants

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Solicitation: NRA

Initial Funding Date: 12/92

Expiration: 5/96

FY 1995 Funding: $60,000

Students Funded Under Research: 5

Task Description:

The overall goal of our research program is to elucidate the gravireceptor and the biophysical events that the activated gravireceptor initiates in order to understand how cells perceive and respond to a gravitational stimulus. In order to accomplish our goals, we have studied the effects of gravity on the polarity of cytoplasmic streaming in the single internodal cells of characean algae.

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 instead, senses 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. As expected, we are continuing to deepen our understanding of gravity sensing in *Chara*. We have also broadened our scope to test this model of gravity sensing in higher plants and fungi.

We have demonstrated that the cell does not sense the gravity vector directly, but senses 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). We have also found that 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. Further, we have 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. Finally, 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 nmol m^{-2} s^{-1} to 60 nmol m^{-2} 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 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 are continuing to characterize the relationship between the activation of the tension and compression receptors and the flux of Sr^{2+}.

While we are primarily using algal cells to understand how cells sense gravity, we hope that this research will have a direct benefit to human beings in two ways: First, we think that the humans are different from animals in the need to understand the world around us. We feel that our research will enhance people's sense of wonder and need to know by helping them understand how cells sense gravity. Why do the shoots of trees grow up and the roots grow down? You are probably sitting down as you read this with your head up and your feet down. Ever wonder how our cells know which way is up and which way is down, even when our eyes are closed? We hope to answer these questions. Second, this research may have a direct benefit to medicine in that the human sense of balance depends on the ability of our cells to sense up and down and respond to the perceived signal. As we age, our sense of balance becomes impaired and this leads to further complications like falling. We hope that if we understand how algal, fungal and higher plant cells respond to gravity, we can apply this knowledge to develop treatments which may help elderly people maintain their sense of balance.

Publications, Presentations, and Other Accomplishments:

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Permeability and Gene Expression in Brain Endothelial Cells Exposed to Shear Stress and Differential Pressure

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Funding:

Project Identification: 199-40-21-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $74,470

Students Funded Under Research: 1

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 cephalid 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.

Ideally, this study would be conducted using human brain microvessel endothelial cells (BMECs). We have established 3 different sources for this tissue (University of Texas Medical Branch, Galveston;

Baylor College of Medicine, Houston; National Tissue Network, Atlanta). However, the supplies of fresh human brains that are not infected with HIV are extremely limited and in high demand. We have successfully isolated brain microvessel endothelial cells from human tissue with only slight modification of our previous procedures used with bovine brains. Unfortunately, the quantities have not been sufficient to conduct the sheer stress studies. As we indicated in our proposal, if human tissue supplies were limiting, we initiated the studies using bovine brain tissue as our source of BMECs.

Studies for our first specific aim, the quantitation of macromolecular permeability and hydraulic conductivity changes in BMECs subjected to shear stress and transmural pressure, have been initiated. Macromolecular permeabilities have been quantitated in bovine BMECs subjected to 1 and 10 dyne/cm2. Ours was the first study to quantitate these shear forces on BMECs for durations of 73 hr. The permeability properties of the cells were quantitated by measuring the flux of fluorescently labeled dextrans that crossed the cell monolayer and the permeability coefficient was calculated. The initial responses to these levels of shear stress resulted in a dramatic increase in permeability, but continued exposure to the shear field resulted in a partial recovery in the permeability of the brain microvessel endothelial cells to macromolecules. Interestingly, the cell exposed to the higher shear forces also underwent a morphological change from a swirling spindoidal morphology to a cobblestone morphology with elongation in the direction of the flow. RNA has been extracted from bovine BMECs that have been exposed to shear stress and their corresponding controls. As we outlined in our proposal, this RNA will be used to generate cDNA libraries. In addition, we are also examining the transcriptional levels of prostaglandin H synthase (PGHS1 and PGHS2) genes resulting after shear stress. The effects of shear stress on PGHS1/2 will be compared in blood-brain barrier endothelial cells (BMECs) and in human umbilical vein endothelial cells (HUVECs) to better understand how physical forces alter cellular function at a molecular level. Initial studies indicate that PGHS2 mRNA levels are increased in HUVECs in response to shear.

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. There ore, 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.

Publications, Presentations, and Other Accomplishments:

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Markers for Assessing Vertebrate Development in Space

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Funding:

Project Identification: 199-40-27-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 1/95
Expiration: 12/97
FY 1995 Funding: $128,244
Students Funded Under Research: 0

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*.

We have made progress in several areas with particular focus on understanding the expression and function of *hsp*70.2, a member of the murine cellular stress protein gene family, in the central nervous system (CNS). Our recent observations provided evidence of the expression of *hsp*70.2 in the brain, in addition to the testis in which its expression is quite abundant. We examined the spatial pattern of distribution of *hsp*70.2 mRNA and protein in distinct regions of the CNS in the presence or absence of exogenous stress, as well as the developmental pattern of *hsp*70.2 expression at the histological level. During the course of this analysis, we made the surprising discovery that both sense (2.7 kb) and antisense (2.8 kb) transcripts from the genomic region of the *hsp*70.2 gene were produced. Given the complex structural organization of the CNS, we were interested to know whether the sense and antisense *hsp*70.2 transcripts were expressed throughout the entire CNS or were spatially restricted. Two experimental approaches were used: i) total RNA was isolated from different dissected regions of the CNS and examined by Northern blot hybridization for the presence of *hsp*70.2 sense and antisense mRNAs, and ii) *in situ* hybridization analysis of the cellular localization of *hsp*70.2 sense and antisense transcripts using both radioactive and non-radioactive RNA probes. Low levels of expression of the 2.7 kb sense transcripts were detected in most areas of the brain, with distinctly higher signal in the hippocampus. The patterns of intensity and distribution of the antisense transcripts were strikingly different: the highest levels were observed in the brainstem, cerebellum, cortex, hippocampus, thalamus and olfactory bulbs. *In situ* hybridization analysis revealed that the expression of both sense and antisense transcripts was associated with neuronal phenotypes in the hippocampus, as well as in the locus ceruleus, the granular layer of cerebellar cortex and the superior olives. In contrast, the patterns

of expression of the *hsp*70.2 sense and antisense transcripts were strikingly different in other regions of the brain with the highest levels of *hsp*70.2 antisense transcripts in the layer of Purkinje cells of the cerebellum, while *hsp*70.2 sense transcripts were not detectable. Abundant *hsp*70.2 antisense transcripts were also observed in the frontal cortex and thalamus; however, sense transcripts were weak or barely detectable in the same structures. Interestingly the sense transcripts were predominantly cytoplasmic and the antisense transcripts nuclear.

One of the issues with which we are concerned is that animals may display different responses to exogenous stress depending on their developmental stage. We therefore asked whether the regional and cellular specificity of the expression of the *hsp*70.2 sense and antisense transcripts changes during embryonic and postnatal neural development. To date, we have begun to examine brains of mice at d 17.5 p.c., d 1, 3, 5- 7 p.n., and d 17 p.n. by *in situ* hybridization. In particular, we made the observation that the expression of the *hsp*70.2 sense and antisense transcripts exhibited a transitory disappearance from the thalamus and weakened in the hippocampus, at days 6-7 p.n., but were again observed on p.n. day 17. These analyses will be extended in the second year of funding, at the level of both RNA and protein. The expression of *hsp*70.2 in adult brain was studied after heat shock treatment and transient CO_2 asphyxia. The methods for *in vivo* hyperthermia represented an improved modification of our originally proposed experimental methodology. Four transient asphyxia animals were subjected to CO_2 treatment for 5 min, let recover, and sacrificed 1 hr after treatment. We observed a decrease in the level of immunostaining in the brain especially in the cortex and hippocampus of the treated animals after both heat shock or CO_2 asphyxia. The observed patterns of *hsp*70.2 expression in the developing brain and after exogenous stress suggest an important role for the *hsp*70.2 gene in normal brain functioning. Finally, our preliminary data from immunoblot analysis of the *hsp*70.2 protein indicated that the molecular weight of the protein expressed in the brain was higher than the protein expressed in the testis. This suggested that the *hsp*70.2 protein in the brain may undergo posttranslational modifications, including glycosylation and/or phosphorylation, which are widespread in the brain.

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 studies 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 on the common man 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.

Publications, Presentations, and Other Accomplishments:

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Performance in Haptic Virtual Environments with Visual Supplement

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Project Identification: 199-06-12-40

Solicitation:

Initial Funding Date: 4/95

Expiration: 3/98

FY 1995 Funding: $249,200

Students Funded Under Research: 1

Responsible NASA Center: Ames Research Center

Task Description:

We propose an applied research and development program to determine human factors guidelines for effective haptic (force reflecting) manual interfaces to multisensory virtual simulator and teleoperation displays. The two major program aspects include: 1) the design and implementation of a novel, very high performance three degree of freedom (dof) force reflecting manual interface for use with our laboratory's virtual visual display as a research testbed; 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) testbed. While prior research has focused on control and system dynamics for manipulation stability in remote and virtual environments, issues specific to successful display of higher bandwidth force reflected sensory information have not received attention. In addition to teleoperation interfaces, application areas for improved force displays include design prototyping and operator training for hand tools and control panels that might be used for in-space assembly, planetary exploration, and scientific data visualization.

We have completed the geometric and kinematic analyses for the novel mechanical linkage that is the foundation of the innovative three degree of freedom force-reflecting haptic interface being developed for this task. The general mathematical description arising from this analysis reveals that the mechanical linkage has a pair of singularity-free hemispherical workspaces suitable for high fidelity control of forces and virtual haptic environments.

The results of the mathematical analysis have been used to determine haptic interface linkage component sizes that provide for a numerically well conditioned operating region that will meet the six inch cube working volume specified in the original proposal. Note that a family of haptic interfaces of different sizes ranging from individual finger up to whole arm devices can now be designed and built based on the general linkage geometry and kinematic analysis we performed. The final linkage embodiment for the haptic interface has been selected based on this analysis and the construction of two unpowered kinematic linkage mock-ups.

Detailed mechanical design, including actuator and sensor selection, is currently in progress. The results achieved to this point do not indicate any significant departures from the proposed research plan. The innovative three degree of freedom mechanical linkage being developed for this task was disclosed in written form to the Patent Counsel at NASA Ames.

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 display in isolation and when combined haptic-visual display is available. Because the study of human haptic interaction and perception of the 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, both on Earth and in space.

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 surgical training, planning, and execution for nearly all parts of the human body.

A plausible space medicine application could employ a computer-controlled haptic interface capable of generating arbitrary force or mechanical dynamics to compensate for strength and muscle changes due to prolonged exposure to microgravity, to counteract the limitations of space flight tools, gloves and suits, or, simply to emulate normal gravity forces on a hand or other body part 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.

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Visual Performance and Fatigue in See-Through Head-Mounted Displays

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Project Identification: 199-06-12-38

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 1/98

FY 1995 Funding: $218,500

Students Funded Under Research: 6

Joint 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 three principle accomplishments of the head-mounted see-through display task in the last year have been 1) The completion of four experiments examining the cause of errors in depth judgments to virtual targets presented via head-mounted displays; 2) The completion of two experiments examining the consequences of monocular, binocular, or stereoscopic viewing on the accuracy of depth judgments of virtual objects (subjective viewing discomfort while using the displays was also studied); and 3) The reduction of measured full system rendering delay for the presentation of head-stabilized, stereoscopic virtual objects from 65 msec to 30 msec and corresponding trebling of average rendering update rate to 60hz. These accomplishments have been reported in refereed proceedings papers and are under consideration for publication in refereed journals.

1) Four experiments have examined a displacement of the judged position of a nearby virtual object associated with the superposition of the object against physical surfaces within arms reach. The experiments measure the extent of the error in depth judgment and show the error is associated with changes in static convergence induced by the presence of the physical surface. The experiments distinguish between purely oculomotor explanations and those based on perceptual interpretation of occlusion. They suggest that shearing of optical contours may be a sufficient stimuli to induce convergence reflexes.

2) Since stereoscopic presentation of virtual objects generally involves rendering for both a left and right image, there is considerable practical gain from examining monocular head mounted displays that may be adequate for many tasks. Studies comparing the accuracy of depth judgments with monocular, binocular, and stereo viewing have shown that for static images with appropriately adjusted convergence planes, binocular displays can present virtual objects with high judgment accuracies comparable to stereo displays. Studies of viewing fatigue while visually tracing space stabilized virtual objects, however, show that binocular viewing conditions produce significant, unique viewing difficulties probably due to conflict between looming and disparity cues to ocular convergence.

3) Excessive end-to-end latency and insufficient update rate continue to be major limitations of virtual environment (VE) system performance. Improved hardware and software reconfigurations have reduced end-to-end latency and increased the update rate. These reconfigurations included: 1) multiple asynchronous UNIX processes communicating via shared memory; 2) continuous streaming rather than polled tracker operation; 3) multiple rather than single tracker instruments; and 4) higher bandwidth IEEE-488 parallel communication between tracker and computer replacing RS-232 communication. Average latency of 65 msec and an update rate of 20 Hz for a standard 1000 polygon test VE, has now improved to 60 Hz (the maximum achievable with our graphics display hardware) with approximately 30 msec average latency. Because our equipment and architecture is based on widely available hardware (i.e., SGI computer, Polhemus Fastrak) and software (i.e., Sense8 WorlToolKit), our techniques and results are broadly applicable and easily transferable to other VE systems.

Results from accomplishments 1 and 2 have been provided to Boeing Computer Services and GM Technical Centers in response to requests. Code from accomplishment 3 will be made available through appropriate NASA distribution systems when requested. Future investigations will examine the role of motion parallax in aiding depth judgments with existing dynamic response and those enhanced by several alternative predictive tracking systems.

Improvements in the full system dynamic response of computer graphic based simulations presenting virtual objects on head-mounted displays (HMDs) are being provided to improve dynamic performance of HMDs to be used for NeuroLab experiments led by Dr. Charles M. Oman of MIT.

Virtual environment displays may provide a new communications medium for spatial information. The research conducted on this current 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, 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 this 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 heretofore impossible 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 since 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 3D 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 in the projects bibliography.

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Behavioral Trends and Adaptation During Space Analogue Missions

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Funding:

Project Identification:

Solicitation: NRA-93-OLMSA-07

Initial Funding Date: 10/94

Expiration: 9/97

FY 1995 Funding: $100,000

Students Funded Under Research: 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:

- Identify and characterize trends in psychological and behavior variables over the course of long-duration analogue missions.
- 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.

Data collection has been completed on two 100-day Australian Antarctic traverses with 6 expeditioners each. These data have been analyzed and a manuscript is in progress. Data from a joint French-Italian expedition were also collected, but response was consistent, and data from that traverse must be added to the data from the other traverses for further analyses. We are awaiting a new statistical package that will facilitate those analyses. Additional data were collected over the FY95 Austral Winter in four

Australian Antarctic stations. Year-round data collection has begun in those same four stations for FY96 and will include all expeditioners, instead of looking only at the wintering parties.

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.

Crew Culture, Selection, Training and Performance

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Funding:

Project Identification: 199-06-17-03

Solicitation: 93-OLMSA-07

Initial Funding Date: 10/94

Expiration: 9/95

FY 1995 Funding: $100,000

Students Funded Under Research: 10

Task Description:

The goals of the project are to investigate the multiple determinants of individual and group performance using a systems approach that includes investigation of national and organizational cultures, group processes, training interventions to enhance group performance, relationships between personality and performance, and analysis of human error in complex environments.

Research during the fiscal year continued the investigation of issues determining team performance in demanding technological environments, primarily using aviation as an analog for space flight. Much of the effort centered on refining methodologies for the assessment of team behavior. The behavioral assessment strategy has been included in the Federal Aviation Administration's Advisory Circular for Crew Resource Management (AC120-51a).

Major findings concerned differences in team attitudes and behavior as a function of national culture. Data from pilots of more than ten nationalities showed highly significant differences in relations between subordinates and leaders and a focus on group rather than individual concerns. These data have implications regarding crew performance in multinational endeavors such as the international space station. In the process of this research, a new instrument to measure work attitudes and values was developed and is being used in the research.

An unanticipated and serendipitous finding from the cross-cultural research was the existence of highly significant national differences in attitudes toward and preference for automation. In general, pilots from many non-Western cultures tend to prefer automation and to rely on it more extensively than do, for example, Americans or Western Europeans. These results may be significant in analysis of air crashes involving highly automated aircraft. A major effort is underway to collect data from additional cultures, including those involved in space station, and to extend measures related to human-computer interaction and automation. Another endeavor involved testing the extension of the team performance and training research to other domains where teams interact with technology - specifically the medical operating room.

Thirty-three articles were published or are in press from FY94 and FY95, including refereed journal articles, chapters, and conference proceedings. In addition, project personnel gave 22 invited papers in

the United States and in the following foreign countries - Bahrain, Ethiopia, Hong Kong, Morocco, the Netherlands, Saudi Arabia, Switzerland, the United Arab Emirates, and the United Kingdom.

The interpersonal processes under investigation in earth analog environments should be comparable in space missions. Interpersonal communication, decision making, conflict resolution, etc. achieve great import when groups are isolated and confined. Hence, any approaches that would enhance teamwork could affect the safety and productivity of missions.

The research has already had an impact on the common man. Training techniques for improving team coordination have been widely adopted in aviation and are being tested with medical teams. The research has validated the impact of training on crew performance and, by inference, has helped increase the safety of commercial aviation. The goal of the present phase of the research is to adapt these strategies to enhancing teamwork among multinational teams, including astronauts.

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Correlation Between Physiology and Shuttle Emergency Egress (1 year proposal)

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Project Identification: 199-26-11-30

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/96

FY 1995 Funding: $8,064

Students Funded Under Research: 0

Responsible NASA Center: Johnson Space Center

Task Description:

An emergency (a 'MODE 5' egress in the operations vernacular) would occur after a landing where the usual ground support crew cannot assist the astronauts in egressing the vehicle. In this study, human test subjects will be trained in performing the egress procedures in NASA Orbiter simulation facilities, then tested in several timed trials. Physiologic properties of the subjects (height, weight, sex, muscle strength, aerobic exercise capacity, orthostatic tolerance, et cetera) and test conditions (weight carried, seating position) will be correlated with test subject performance in the trials as measured by completion times and by O_2 consumption. It is hypothesized that lower strength and fitness levels observed in the test subjects will correlate with lower performance (higher completion times and higher O_2 consumption) during egress.

The final result of the project will include 1) an assessment (mean and standard deviation) of the time and O_2 supply required for emergency egress and a correlation of those data with key physiologic parameters thus proving or disproving the hypothesis; 2) an identification of critical human performance difficulties to guide future countermeasure development; 3) verification of the 1988 bailout bottle sizing estimate; and 4) a recommendation for future research directions.

Perceptually-Tuned Visual Simulation

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Funding:

Project Identification: 199-06-12-05

Solicitation: 93-OLMSA-07

Initial Funding Date: 5/95

Expiration: 4/98

FY 1995 Funding: $158,000

Students Funded Under Research: 5

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 propose 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 will automate 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 3-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.

During the first eight months of the task, we have made a number of significant accomplishments, most notably the patent application for variable resolution rendering. In addition, we have pursued research on the most effective techniques for level of detail modulation, and evaluated the efficacy of billboarding techniques for database simplification. Two graduate students working on the project have completed their dissertations on programmatic research; one is staying on the project as a National Research Council Postdoc, the other has taken a position on the technical staff of Silicon Graphics, Inc. SGI continues to be one of our more promising industry liaisons, although we have also had

useful meetings with Sun Microsystems, Hewlett-Packard, Interval Research, Division Inc., and Amtech.

Results in our laboratories at Ames and University of Virginia and feedback from industry experts indicate two new issues our research needs to address: 1) a method to create generalized 3-D object meshes to permit polygon phase-shifts; and 2) a technique to avoid color aliasing when certain colors are overlapped in the phase-shift.

We are also working to develop a general method to extend the most promising level of detail transition technique, morphing, to three-dimensional objects. User evaluation studies are ongoing to refine hi-low stereo displays and examine their efficacy (and test for any undesirable artifacts or after-effects).

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.

Publications, Presentations, and Other Accomplishments:

Kaiser, M. K. "Supercomputers and visual simulation." 25th Annual Meeting of the Society for Computers in Psychology, November, 1995.

Kaiser, M. K., Montegut, M. J., & Proffitt, D. R. "Rotational and translational components of motion parallax: Observers' sensitivity and implications for 3-D computer graphics." Journal of Experimental Psychology: Applied, 1, 321-331 (1995).

Kaiser, M. K., Sweet, B. T., & Shafto, M. "Depth cue integration in an active control paradigm." 36th Annual Meeting of the Psychonomic Society, November, 1995.

Proffitt, D.R. "Human Factors in Virtual Reality Development." Annual meeting of SIGGRAPH, August, 1995.

Patent In Process, U. S. Patent #: Undetermined, Proffitt, D.R. and M.K. Kaiser. "Spatial-Temporal Resolution Process for Computationally Efficient Displays."

Crew Behavior and Performance in Ground Operations

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Funding:

Project Identification: 199-06-12-04

Solicitation: 93-OLMSA-07

Initial Funding Date: 5/95

Expiration: 4/96

FY 1995 Funding: $112,000

Students Funded Under Research: 2

Joint Participation: DoD

Responsible NASA Center: Ames Research Center

Task Description:

Ground operations in support of space flight require error-free precision work by technicians in payload, shuttle, and space station operations. However, due to time pressures, complexity of the working environment, dynamic changes in schedules, introduction of new technologies, etc., human error problems can and do occur. With anticipated cutbacks in personnel and other resources, careful assessment of human performance requirements are more important than ever.

There is not a long history of human factors research in ground operations, nor is there a readily available archival database of operational knowledge that points to the most critical problems, their underlying issues, and "lessons learned." In many cases, teams learn to "live with" procedures and practices that are cumbersome but safe. In time and with experience, coping strategies are learned on the job. In other cases, teams create innovative improvements that enhance safety, productivity, and job satisfaction, but these solutions are seldom extended or standardized for benefit outside the group. Thus, we propose to focus on two main objectives: 1) the identification of crew factors safety problems, as well as effective information and team management strategies, and 2) the development of more systematic approaches toward documenting and assessing human performance requirements, and in implementing training, procedural, and technology solutions to human performance problems.

The goals stated above have been formulated into 2 focus areas: 1) human factors training including the KSC task team leadership program, and 2) process analysis techniques for the systematic analysis of human error incidents and events. In the two general areas above, we have provided several briefing and workshop opportunities for the Human Factors teams at Kennedy Space Center (including both NASA and Lockheed/Martin representatives). During April and June we gave informal briefings on incident analysis techniques, human factors training and enhancing team performance at KSC. During September, we conducted a 3-day leadership workshop at the Center For Creative Leadership in

Colorado Springs. In addition to general consultative briefings and workshops, we completed our research based upon two field data collection efforts. These efforts took place in Shuttle Operations at Kennedy Space Center during the summers of 1993 and 1994. Data analyses and results were completed during FY95 and incorporated into the briefings described above.

Because there is much overlap in human factors problems and issues across other high risk, complex work environments, we conducted an analysis of incident data from the aviation maintenance domain. The NASA Aviation Safety Reporting System provides a rich archive of actual operational events which are related to maintenance procedures and processing. Because such a database is lacking in the aerospace community, the analysis was undertaken as both a model for shuttle operations and for generalizable substantive results.

When one studies the underlying processes, procedures, training issues and even errors made, the commonality of human factors issues across many high-risk complex, and sometimes hazardous work environments become obvious. Such domains include aviation, ground and maritime transportation systems, the nuclear power industry, chemical and other manufacturing plants, and many other safety critical workplaces.

While there are special concerns in each domain, work related to human factors, human error, risk analysis, team training, performance metrics, etc. can be easily shared and adapted. Our approach in working with KSC operations is to bring in expertise from other areas, adapt "lessons learned" to shuttle operations when appropriate, and finally, to conduct field specific research at KSC which has direct relevance to operations and which can be easily generalized to other high-risk complex environments. Technology transfer and information sharing is a basic and necessary foundation for this type of operational research to be most effective.

Publications, Presentations, and Other Accomplishments:

Kanki, B.G., Prinzo, O.V., and Schiflett, S. (Eds.) Proceedings of the Methods and Metrics of Voice Communication Workshop, San Antonio, TX (in press).

Lieberman, P., Kanki, B.G., Protopapas, A., Youngs, J.W., and Reed, E. "Speech production and cognitive defects on Mount Everest." Aviation, Space and Environmental Medicine, vol. 66, no. 9, 857-864 (1995).

Lieberman, P., Protopapas, A., Reed, E., Youngs, J.W., and Kanki, B.G. "Cognitive defects at altitude." Nature, vol. 372, no. 6504, 325 (Nov. 24, 1994).

Robertson, M.M., Taylor, J.C., Stelly, J.W. Jr., and Wagner, R.H. Jr. "Maintenance CRM training: Assertiveness attitudes and maintenance performance in a matched sample." Proceedings of the 21st Conference of the Western European Association of Aviation Psychology (in press).

Taylor, J.C., and Robertson, M.M. "The effects of crew resource management (CRM) training in airline maintenance: Results following three years' experience." NASA Tech Brief, NASA CR 196696, (1995).

Walter, D., and Kanki, B.G. "A human factors approach to aviation maintenance and inspection training: The Task Analytic Training System." Proceedings of the 8th International Symposium on Aviation Psychology, Columbus, OH, Ohio State University (in press).

An EVA Strength and Reach Model

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Funding:

Project Identification: 199-06-11-46

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/94

Expiration: 2/97

FY 1995 Funding: $92,900

Students Funded Under Research: 2

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, strength and fatigue capabilities. Mission planners could use the modeling system and view the animations and the visualizations of the various parameters, such as overall motion, reach, fatigue and strength to streamline the timing, duration, task arrangement, personnel and 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, it will require collecting EMU suited strength, range of motion, and fatigue data for all the major suit joints. The suit dimension measurements and joint limit data will be the basis for building the suit in the graphics environment. 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 (NASA Technical Papers 3206, 3207). Motion analysis data along with collected multi-joint motion torque data will be used to validate the EMU kinematic and strength model.

Strength Data collected on 6 EMU suited subjects was processed and analyzed for the wrist, elbow, shoulder, and knee for five different test conditions. The conditions were as follows: 1) unsuited, 2) Class 1 EMU suited, 3) Class 1 with TMG (Thermal Meteoroid Garment) EMU suited, 4) Class 3 EMU suited, and 5) Class 3 with TMG EMU suited. Statistical methods are being used to look for analytical differences between conditions.

Software has been developed to read a Cyberglove (18 Dof) and has been integrated with a graphical model of a hand. Software was also developed to integrate a hand dynamometer to collect hand strength information at the same time that the Cyberglove collects kinematic information. A strength and motion test was performed which utilized a pressurized EMU glove, the Cyberglove (for kinematic information), and a hand dynamometer (to collect grip strength data). Data from this test is being analyzed and will be incorporated into a hand strength model.

Hardware modifications on the LIDO Multi-joint II dynamometer have begun. These modifications will allow the collection of strength data for the hip and ankle joints of the EMU. The schedule for completion of this modification has been moved due to the scheduling conflicts within the ABL with their yearly astronaut candidate measurement review. This reschedule should not impact the current ongoing data and modeling activities other than the work to be performed by Dr. Badler's group (see below).

A test plan to collect the next stage of EMU strength data has been developed. This plan details the protocol of EMU strength data collection. The data collection will be scheduled as soon as the additional needed LIDO attachments are built and tested. Strength Data for 15 unsuited subjects were analyzed for wrist, elbow, shoulder, hip, knee, and ankle isolated joint strengths. This data was reduced into regression coefficients.

Modeling:
Software has been developed which allows the creation of an EMU graphical model from given anthropometric data. This model is fully articulated and constrained by joint limits. Validation tests on the accuracy of the anthropometric model are underway. A postural analysis software package has been developed for modeling EMU posture. This model utilizes collected strength data to evaluate motions of an EMU task. It uses motion data gathered to analyze preferred postures. The theory is that comfort levels are a function of isolated joint strength measures. The model is currently being verified.

Dr. Badler's group at the University of Pennsylvania is to assist the project with detailed modeling of certain aspects of the EMU suit, especially those pertaining the modeling of the kinematics. However, due to scheduling problems related to suit access and modifications of hardware for the collection process, this originally scheduled 1995 task will take place in FY1996. Synchronization of schedules and specifics for the statement of work are being determined at this time. The funding for this particular activity is already in place.

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.

Publications, Presentations, and Other Accomplishments:

Morgan, D., A. Pandya, R. Wilmington, J. Maida and K. Demel. "Toward modeling space suited joint strength." American Society of Biomechanics Annual Meeting, August 1995.

Human Interaction Design for Cooperating Automation

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Funding:

Project Identification: 199-06-11-50

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/94

Expiration: 2/97

FY 1995 Funding: $105,000

Students Funded Under Research: 1

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 task analysis methodology to support the level of analysis needed to design intelligent automation systems to be "team players". Project products will include reusable designs, guidelines and methods.

In FY95, initial methods for task analysis and scenario generation for intelligent automation systems were documented in NASA Technical Memorandum 104807, Making Intelligent Systems Team Players: A Guide to Developing Intelligent Monitoring Systems. Methodology was also documented in a section on intelligent cooperative systems in a chapter on intelligent interfaces, to be published in the forthcoming new edition of the Handbook of Human-Computer Interaction, and in the initial draft of a Human Interaction Design Field Guide. Human interaction design has begun on intelligent software for situation capture and reporting to support anomaly response for Mechanical Maintenance and Crew Systems (MMACS) flight controllers. A variety of observational, interviewing, and prototyping methods have been applied to understanding the tasks and task context for this type of intelligent automation function. Some preliminary design concepts have been generated for situation capture and reporting, based on previous work with the Remote Manipulator System (RMS) Decision Support System (DESSY), a Mission Control Center intelligent system.

Benefits to medical applications and industry 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 and examples and the task description

methodology will advance human factors engineering knowledge and practice for complex multi-screen multi-application operations support systems.

Publications, Presentations, and Other Accomplishments:

Land, S.A., J.T.Malin, C.Thronesbery and D.L. Schreckenghost. "Making intelligent systems team players: a guide to developing intelligent monitoring systems." NASA-Johnson Space Center, Houston, TX, NASA Tech Brief, 104807, (1995).

Malin, J.T. and C.G. Thronesbery. "Application reuse library for software, requirements and guidelines." Dual Use Space Technology Transfer Conference and Exhibition (NASA Conference Publication 3263). NASA-Johnson Space Center, Houston, TX, 203-210, February, 1994.

Malin, J.T., D.L. Schreckenghost and C.G. Thronesbery. "Principles and methods for design of adaptive cooperative automation, Book of Abstracts." Life Sciences and Space Medicine Conference '95. AIAA and NASA, Houston, TX, 186-187, April, 1995.

Perceptual Optimization of Image Compression and Displays

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Funding:

Project Identification: 199-06-12-39

Solicitation: 93-OLMSA-07

Initial Funding Date: 4/95

Expiration: 3/98

FY 1995 Funding: $261,200

Students Funded Under Research: 7

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.

The major accomplishment during FY95 has been the completion of psychophysical experiments to determine human visual sensitivity to wavelet basis functions and quantization error. These data, and the mathematical model derived to explain them, provide a new principled basis for perceptual tuning of wavelet compression schemes. This work has been reported in a journal article and three conference presentations. In the next phase of this work we will insert the perceptual model into a wavelet compression standard and evaluate performance. This work will be undertaken in collaboration with Los Alamos National Lab. We will also commence work on perceptual compression of moving images (video) in the context of the MPEG algorithm.

In related work in perceptual image compression, we collaborated with authors from the Institute for Perception Research, Netherlands and Cedars Sinai Medical Center, received a US Patent for a perceptual compression technique, and published a report on a DCT smoothing technique.

In applied work on developing vision models of display optimization, we have been able to show the utility of simple discrimination models in predicting detection of objects in natural scenes. These models will form the foundation for further development of tools for optimization of compression and displays. During the coming year, we will be particularly interested in whether simple efficient variants of these models can achieve comparable results.

Our applied research is built upon a foundation of basic research. During the past year we have made progress on models of spatial vision, motion perception, and eye movements. In spatial vision, we have developed and validated a general computational model of contrast gain control in human vision. This is the system that adapts the eye to ambient levels of contrast, thus preserving its limited dynamic range, but thereby producing various powerful masking effects whose understanding is essential to optimization of compression and displays. Research on autonomous calibration of the visual system was also reported. In the area of motion perception, we published measurements characterizing the fundamental human visual motion sensor, and collected and modeled data characterizing the spatial and temporal pooling of motion signals. In the area of eye movements, which are themselves crucial to motion perception and to the perception of artifacts in compressed video, we completed work on a new system for video recording of fundus image motion, and experiments characterizing the relation between eye movements and motion perception.

In support of our psychophysical work, we have developed software tools for easy generation of calibrated displays on personal computers. In a small but intriguing consultation, Dr. Watson advised a NASA astronomer on how to predict the apparent color of Saturn's rings from radiometric data. As a service to the vision, human factors, and imaging communities, we have developed and continue to maintain a world-wide-web page of resources in Vision Science (http://vision.arc.nasa.gov/VisionScience), which receives over 10,000 hits per week.

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, to the world-wide-web, to high-definition television, to virtual reality, to 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.

Publications, Presentations, and Other Accomplishments:

A. B. Watson and J. A. Solomon "Contrast gain control model fits masking data." Investigative Ophthalmology & Visual Science, vol 36, S438 (1995).

A. B. Watson and K. A. Turano "The optimal motion stimulus." Vision Research, vol 35, 325-336 (1995).

A. J. Ahumada, Jr., A. B. Watson, and A. M. Rohaly "Models of human image discrimination predict object detection in natural backgrounds." Human Vision, Visual Processing, and Digital Display VI (IS&T/SPIE), San Jose, CA, 1995.

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A. J. Ahumada, Jr. and B. Beard "(Abstract) Image discrimination models with stochastic channel selection." Optics and Photonics News, vol 6, 138 (1995).

A. J. Ahumada, Jr. and C. H. Null "Image discrimination predictions of a single channel model with contrast gain control." Psychonomic Society, Austin, TX, 1995.

A. J. Ahumada, Jr. and K. Turano "Exploratory Vision: The Active Eye (contribution: Calibration of a visual system with receptor drop-out)." Edited by: L.T.M. Michael Landy and Misha Pavel Springer-Verlag, New York, pp 157-168, 1995.

A. M. Rohaly, A. J. Ahumada, Jr., and A. B. Watson "A Comparison of Image Quality Models and Metrics Predicting Object Detection." Society for Information Display International Symposium Digest of Technical Papers, vol. 26, J. Morreale, Ed. Santa Ana, CA:,Society for Information Display, pp. 45-48, 1995.

B. R. Beutter, J. B. Mulligan, and L. S. Stone "Analysis of the trial-by-trial correlation between eye movement and perceptual responses to moving plaids." Society for Neuroscience Abstracts, vol 21, 141 (1995).

J. A. Solomon and A. B. Watson "(Abstract) Spatial and spatial frequency spreads of masking: measurements and a contrast gain-control model." Perception, vol 24, 37 (1995).

J. B. Mulligan "Temporal MTF of motion perception." Optical Society of America, vol. 1995 Annual Meeting, 148, 1995.

J. B. Mulligan and B. R. Beutter "Eye-movement tracking using compressed video images." Vision Science and its Applications, 1995 OSA Technical Digest Series, vol 1, 1163-1166 (1995).

J. B. Mulligan, B. R. Beutter, and S. B. "Stevenson Reflexive OKN is biased like perception." Inv. Ophth. Vis. Sci. (suppl.), vol 36, 205 (1995).

M. Eckstein, C. A. Morioka, J. S. Whiting, and N. Eigler "Psychophysical evaluation of the effect of JPEG, Full-frame DCT and Wavelet image compression on signal detection in medical image noise." International Society of Optical Engineering Annual Meeting, Medical Image Perception, 1995.

M. Pavel, R. Sharma, and A. J. Ahumada, Jr. "(Abstract) Masking by fixed and random noise." Optics and Photonics News, vol 6, 64 (1995).

R. Horng and A. J. Ahumada, Jr. "A Fast DCT Block Smoothing Algorithm." Visual Communication and Image Processing '95, Proceedings Volume 2501, L. T. Wu, Ed., Bellingham, WA, SPIE, pp. Paper 5, 1995.

T. van Dijk, J.-B. Martens, and A. B. Watson "Quality assessment of JPEG-coded images using numerical category scaling." European Symposium on Advanced Networks and Services, Amsterdam, The Netherlands, 1995.

Watson, A.B. "Image data compression having minimum perceptual error." US: The United States of America, 1995.

Human Interaction Design for Anomaly Response Support

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Funding:

Project Identification: 199-06-17-01&199-11-11-52 Solicitation: 93-OLMSA-07

Initial Funding Date: 4/95 Expiration: 3/98

FY 1995 Funding: $100,000 Students Funded Under Research: 1

Task Description:

We are developing computer support concepts and systems for aiding cooperative anomaly response. To do this, we are conducting a cognitive analysis of anomaly response activities in space shuttle mission control. This analysis focuses on the coordination that occurs across interdependent teams when subtle problems arise in space shuttle systems. Currently we are studying the anomaly response activities of a team of controllers who monitor the mechanical and crew systems on the space shuttle. We are analyzing the interactions between this team and other interdependent teams who become involved when anomalies arise. We are studying how these teams' perspectives and knowledge overlap in ways that minimize the chances for errors in the anomaly response process. We will combine the information gained from our analyses with principles of effective human computer interaction to produce computer design concepts for useful tools to support anomaly response when it is distributed across interdependent teams. These design concepts should allow NASA to develop effective anomaly response support tools as the mission control structure changes to adapt to shrinking resources.

Products will include a general model of the cognitive processes involved in distributed anomaly response, a detailed description of anomaly response in the space shuttle mission control domain, prototypes that will allow us to explore computer aiding concepts for supporting anomaly response, and generic design concepts which will support the development of anomaly response aids in cooperative domains.

To date, we have conducted observations, interviews, and reviews of past anomaly cases. Currently, we are focusing on the MMACS flight control team, who is responsible for the shuttle mechanical and crew systems. We have analyzed and observed how the MMACS team coordinates with related flight control teams and other ground support groups like the Mission Evaluation Room (MER), who become involved as the anomaly response process escalates. We observed the MMACS flight control team during training simulations and missions and 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 to further investigate the activities necessary for anomaly response. Our activities have studied how members of the MMACS team and the MER, as well as other ground support groups, cooperate and coordinate activities to successfully handle anomalies.

Specifically, we have examined how these teams cooperate to minimize the chance for errors in the anomaly response process. Our activities have allowed us to develop a framework for our cognitive model, as well as ideas for aiding concepts to support anomaly response, especially as staffing levels change. We have also created an initial prototype to explore some of these aiding concepts. Our model of anomaly response will grow and evolve as we continue to develop and evaluate prototypes based on our aiding concept ideas.

Our research will benefit the space domain by maintaining the high level of performance in mission operations by supporting increased efficiency and productivity across teams who coordinate their efforts to respond to anomalies. Cooperative anomaly response occurs in many different domains. Our understanding of cooperative anomaly response and our computer support ideas will transfer to other domains like air traffic management, nuclear power, and anesthesiology, in which interdependent teams coordinate to respond to anomalies.

Adaptive Visual-Vestibular Mechanisms and Gravity

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Funding:

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Students Funded Under Research: 0

Task Description:

Throughout the history of the manned space flight program, the introduction of the body into microgravity has produced vestibular-related symptoms that result in personal discomfort and a loss in crew performance. Since the symptoms subside within several days of microgravity exposure, it suggests that interactive visual-vestibular mechanisms may be responsible for the initiation of symptoms and their 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. The proposed 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 though 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 a 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, efforts have focused on the following goals: First, to assemble the three-dimensional turntable, order the required equipment and make the PI's laboratory fully functional. Second, to complete on-going work on topics directly relevant to the Specific Aims of the grant. Specifically, based on experiments during off-vertical axis rotations, we have demonstrated that there are three distinct aspects of the otolith-ocular system: 1) Otolith-ocular reflexes appropriate to stabilize gaze during translational motion. These reflexes constitute together with the semicircular canal-ocular reflex the traditional vestibulo-ocular reflexes whose function is to provide robust, short latency oculomotor responses during any combination of linear and angular movements. 2) Direct gravity effects (coding head orientation relative to gravity) on both fast and slow eye movements that appear to dynamically modulate Listing's coordinates and primary eye position as a function of head orientation in space. This reflex is responsible for previous observations described as ocular counter-rolling and "tilt" otolith-ocular reflexes. 3) An inertial vestibulo-motor system detecting angular motion of the head in space. This system processes vestibular signals based on the unique spatio-temporal pattern of mutually perpendicular gravitational and jerk signals which are associated with angular head movements relative to gravity (consequently, it is not activated during linear translation). A distinction and a clear separation between these three distinct aspects of the otolith-ocular system is absolutely necessary before we understand the adaptive processing of three-dimensional gravity signals. The next goal is to investigate how these different gravity-controlled responses are adaptively modified under altered visual/vestibular conditions. Understanding the principles which govern such adaptive visual/gravity interactions are important and critical for dealing with motion sickness and spatial disorientation associated with space flight.

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.

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Neural Plasticity: Data and Computational Structures

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Students Funded Under Research: 38

Joint Participation: NIH and Human Brain Project

Task Description:

This program project combines multilevel research on mechanisms of neural plasticity in basal ganglia, hippocampus, and cerebellum with the development of new informatics tools which will be (a) tested by their ability to stimulate, integrate, and disseminate this neuroscience research at USC, and then (b) developed into user-friendly versions of these new database, visualization, and simulation tools. These tools will be released to the broad neuroscience community, and will integrate and catalyze the development of basic research in neuroscience. Our informatics work builds on four existing projects at USC: (i) research on the construction of object-oriented databases, together with database communication/integration mechanisms and discovery tools; (ii) vector-based modeling of anatomical structures of the rat brain; (iii) pixel-based functional imaging of the human brain; and (iv) our internationally used Neural Simulation Language, NSL, which provides an object-oriented methodology for neural simulation. We will develop an integrated, easy to use, environment in which neuroscientists can store, visualize, retrieve and model complex data sets at all levels of detail. Research and development of this new informatics methodology will proceed in tandem with - both contributing to, and being tested by - the gathering of new experimental data (neurochemical, neurophysiological, and neuroanatomical) and the construction of computer models, for mechanisms of neural plasticity in learning (studied in hippocampus and cerebellum) and in compensation of disease (studied in basal ganglia), paying special attention to the integration of analyses of circuit properties and synaptic mechanisms. Three integrated, commonly housed Cores - Data Base Services, Visualization, and Simulation Tools - will provide technology transfer for tools developed in our informatics research. This research, in turn, will be strongly influenced by feedback obtained as the Core Services are used in basic research on neural plasticity. We are integrating research at seven different laboratories for neuroscience experimentation in three different buildings on USC's two campuses; this will provide the basis for scaling up to a database/visualization/simulation environment

that will meet the central aims of the Human Brain Project. The result will be twofold: continuing progress in a multilevel neuroscience research program on learning and compensation for disease to yield a set of exemplary databases as a nucleus for broad-scale database construction; and computer science research which will yield new user-friendly database/visualization/simulation tools for dissemination to neuroscience laboratories worldwide.

Evaluation of database and interface requirements for the Thompson, Berger, and Arbib Laboratories and the analysis and design of the user interface have been completed. In addition, prototype implementation is in progress.

We have studied how best to summarize the collection of an information repository, based on latent semantic indexing (LSI) of bibliographic descriptions and evaluated the scheme for use in upper level Internet Web indexers, such as Harvest, Lycos, and Yahoo.

To help catalyze the integrated development of experiment and modeling in the neuroscience community, the USC Brain Project is developing Brain Models on the Web. This will include a set of exemplary neural models consisting of memory and behavior studied at the network level; neural plasticity studied at the synaptic and kinetic levels; and models which integrate across the levels. The current version of Brain Models on the Web contains a number of models written in versions 2.1 and 2.5 of our neural simulation language, (NSL).

The goal of the visualization core is to develop a computer-based graphical resource for the display and analysis of functional and structural neuroanatomy. The graphical resource will be integrated with the other core programming efforts to generate an integrated approach to database management, data modeling and data visualization.

During year 1, a visualization tool was placed on the Internet for general access. The initial version allows users to request 2-dimensional sections in sagittal, orthogonal or sagittal orientation from 3 dimensional volumes. 3 Dimensional volumes were initially derived from human MRI data.

We utilize a human CNS flat map to analyze findings on connectivity and 3D imaging in uniform "overview" to aid data integration. We devised tools to register *in situ* sliced rat brain (which is in digital format) in same plane as Swanson rat brain Atlas. This will allow estimation of distortion in the latter, because there is almost none in the Toga brain, and continued work on the production of a digital Atlas of the developing rat brain, to complement the adult Atlas already published.

The overall goal of this project is to develop a research database that: 1) supports the role of the basal ganglia in the coordination of arm and hand movement, and 2) becomes the basis for the formation of a computational model of basal ganglia function with the capacity to explain and predict behavioral deficits seen in the control of voluntary arm and hand movements. Year one goals included: developing the structure of a hierarchical object-oriented database of neurobiological data on the basal ganglia, reflecting and identifying both accepted and controversial data; developing database links between neurobiological data and computational models of the basal ganglia based upon experimental data; providing tools for the execution of the computational model from within a WWW browser; and implementing two neural-network models - the Dominey-Arbib Saccade Model and the Bischoff Basal Ganglia Model - for WWW browsers.

The Cerebellum Group has developed a database for abstracting, storing and retrieving of neurophysiological/anatomical and behavioral data on the role of the cerebellum in classical conditioning of discrete responses [with database group]. Based on these data, the Group developed a detailed computational model of the functions of the cerebellum in learning, memory and adaptive behavior [with Arbib group]. The Cerebellum Group has been involved in the study and model biochemical mechanisms of long-term depression in cerebellum and hippocampus and the study and model integration of cerebellum and hippocampus in trace conditioning.

During the first year of support, the Hippocampus Research Group focused in part on formulation of a relational schema that would provide part of the foundation for the data model. A relational schema was proposed and was evaluated with respect to several data sets generated by standard experimental protocols involving electrophysiological analysis of synaptic transmission using *in vitro* slice preparations. Currently the proposed schema is being revised to be more generally applicable to data collected with other experimental paradigms.

In addition to developing the relational schema, the Hippocampus Research Group made major progress on theoretical issues required for the development of appropriate models of synaptic plasticity. LTP/LTD Group has integrated with Visualization Core to incorporate autoradiographic, immunocytochemical and other histological maps with digital maps.

Based on results from the mappings, we established quantitative maps of NMDA and AMPA receptors in hippocampus and cerebellum. We also interact with Information Management Group to generate Databases of Glutamate Receptors, and Simulation Group to integrate receptor data into hippocampal and cerebellar networks.

This research is primarily aimed at neural mechanisms of change (plasticity) in the nervous system. We thus study both learning (in cerebellum and hippocampus) and compensation for disease (Huntington's disease and Parkinson's disease of the basal ganglia). The research is Earth-based, but will not only yield neuroscientific insights, but will yield informatics tools for integrating experimental data, visualization tools and simulation tools to foster integration of modeling and experimentation - providing tools and a methodology that will be of value in a wide variety of NASA missions both on Earth and in space. The work will make it easier for both scientists and "the common man" to access via the World Wide Web a wide variety of current data and models of brain function.

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Adrenoreceptor Hypersensitivity in Models of Weightlessness

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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: bed rest 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 bed rest on sympathetic function. Our preliminary studies also provide evidence of hyperresponsiveness of adrenergic agonists in patients with orthostatic intolerance. These patients have a clinical picture similar to that observed in astronauts upon return to 1g.

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: bed rest deconditioning, and patients with orthostatic intolerance.

In preparation for our bed rest study we believed it important to develop state of the art techniques to assess sympathetic function. Plasma NE is commonly used as an indicator of sympathetic function but plasma NE not only reflects NE release from sympathetic nerve terminals, but is also influenced by the rate of NE clearance. An increase in plasma NE could be due to a decrease in NE clearance rather than to an increase in NE release. The magnitude of this problem is exemplified by our pilot studies during lower body negative pressure (LBNP). This procedure induces a significant increase in arterial and venous norepinephrine. However, this increase in plasma norepinephrine is in part due to a reduction in norepinephrine clearance due to a decrease in cardiac output. The increase in plasma norepinephrine, therefore, overestimates the actual magnitude of sympathetic activation induced by LBNP. This can be more accurately determined by measuring norepinephrine spillover. This technique is now established in our laboratory and will be used for the first time in space-related research.

Orthostatic intolerance is the most common form of autonomic dysfunction. It produces significant disability with a clinical picture that resembles that observed on astronauts upon return to 1g. We

studied adrenoreceptor sensitivity in 12 patients with orthostatic intolerance and 10 controls. Patients were hypersensitive to the tachycardiac effects of isoproterenol (b1 receptor function) and the pressor effect of phenylephrine (a1 receptor function). No difference in sensitivity was observed to nitroprusside injections (non-adrenergic control).

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 results indicate that these patients have increased responsiveness to adrenergic agonists. It is noteworthy that they also have a significant increase in plasma norepinephrine. The normal physiological response to this increase in circulating catecholamines is a down-regulation of adrenergic receptors, rather than the apparent up regulation observed in our patients. It is possible, therefore, that adrenoreceptor hypersensitivity contributes to the pathophysiology of this disease. It is this hypothesis that we plan to test in the next year of support.

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Otolith and Vertical Canal Contributions to Dynamic Postural Control

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Task Description:

The objective of this project is to determine the role of otolith and vertical semicircular canal vestibular receptors in normal and abnormal human dynamic postural control. This project addresses the following questions: 1) How do normal subjects adjust postural movements in response to changing or altered otolith input, for example, due to aging? and 2) How do patients adapt postural control after altered unilateral or bilateral vestibular sensory inputs such as ablative inner ear surgery or ototoxicity, respectively? We are investigating the following hypotheses: 1) Selective alteration of otolith input or abnormalities of otolith receptor function will result in distinctive spatial, frequency and temporal patterns of head movements and body postural sway dynamics, and 2) subjects with reduced, altered or absent vertical semicircular canal receptor sensitivity but normal otolith receptor function or vice versa, should show predictable alterations of body and head movement strategies essential for the control of postural sway and movement. The effect of altered postural movement control upon compensation and/ or adaptation will be determined. Our hypotheses also predict that intact vertical canal and otolith function in an only remaining ear will permit the human brain to interact visual, vestibular and somatosensory function for restoration of normal postural control. These experiments will provide data for the development of computational models of postural control in normals, vestibular deficient subjects and normal humans exposed to unusual force environments, including orbital space flight.

Results so far have supported the hypothesis that abnormal subjects with otolith function intact in one ear can interact vision, foot contact and residual vestibular inputs for control of anterior-posterior postural sway. We have demonstrated, for the first time, that a subject with an intact superior division of the vestibular nerve, but sectioned inferior division in one ear and sectioned superior division of the vestibular nerve but intact inferior division in the other ear, can successfully " spatially integrate" information from the two ears to control posture in the absence of accurate visual and somatosensory information. In collaboration with Dr. Bill Paloski, JSC, we completed a study of pre- and post-flight postural control in astronauts. Among other things, we have quantified, for the first time, the adaptive control effects of reduction and re-introduction of gravitational otolith inputs on postural stability. The relative roles of visual and proprioceptive inputs during recovery of normal postural control upon return from orbital flight have been characterized. The effects of previous experience upon post-flight ataxia and recovery have also been defined. Our results suggest that the early recovery is dominated by

adaptive control mechanisms, but the reduced severity of post-flight ataxia and faster recovery are consistent with the concept of "efferent copy". These results suggest that pre-flight postural stability during sensory organization tests predicts post-flight performance.

The question of how the adaptive control systems work during active versus passive movements remains to be addressed. An understanding of how sensory feedback modifies movements in microgravity where linear accelerations are induced primarily by active movements remains to be defined. Such passive movement induced "conflicts" to the brain are probably the major stimulus for space motion sickness. An understanding of these processes are essential if progress is to be made in developing robust countermeasures for the negative effects of microgravity on the neuromuscular and cardiovascular control systems. The use of artificial linear accelerations for countermeasures likely will not be possible until this problem is solved.

Results to date confirm our impressions that we must have a better understanding of the multi-dimensional effects of linear accelerations on the human vestibular and converging sensory systems, and how interactions of these systems are affected by microgravity environments. Results from these experiments will help prepare for critical experiments on the long term effects of microgravity on space station crew members.

Vestibular disorders are very common, affecting approximately 90 million Americans over their lifetime. Loss of work and disability due to vertigo, imbalance and spatial disorientation is thought to be very costly. Vestibular disorders are a common cause for falls in the elderly. Spatial disorientation results in significant loss of life and equipment in the military each year. Results of NASA sponsored vestibular research have produced the only large N normal data bases and have contributed significantly to our understanding to vestibular adaptive and compensation processes.

This research seeks to develop new therapeutics or protocols for alleviating symptoms of a disease or malady on earth, from mal de debarquément following cruises which are very disabling for the elderly, to Meniere's disease, to recovery from acute vestibular insults, to avoidance of ototoxicity, to common motion sickness; NASA sponsored research has brought us much closer to diagnostic methods and treatment of these debilitating problems.

The mechanisms of sensorimotor adaptive control of human posture and movement are being characterized. For example, we have begun to characterize the minimal vestibular input, as a function of frequency and amplitude, required for dynamic visual stabilization in the vestibular deficient human.

The only environment in which all inputs to the vestibular system can be removed and re-introduced systematically is in space. The fundamental conditions, in the strict sense, for scientific investigation of vestibular mechanisms therefore require the microgravity environment of space flight. Our work expands upon the classical studies of vestibular mechanisms and, through collaboration with NASA scientists and other colleagues performing ground based research, provides the terrestrial data base (and possible technology) for support of space research in humans.

In combination with support from NIH and collaboration with other scientists, we have developed new diagnostic and therapeutic methods for patients with vestibular disorders. The postural EquiTest postural control (computerized dynamic posturography) assessment system was featured at recent jointly sponsored NIH/NASA exhibit in Washington, D.C. at the Hart Senate Office Building. Our team has developed a new surgical technique for the repair of perilymph fistulas (fluid leak from the inner to the middle ear). Our laboratory published the first large N data base of vestibular function tests (both vestibulo-ocular and vestibulospinal) in the same human subject, and are in the process of testing the same subjects a decade later for the first longitudinal study performed in humans.

The postural control assessment system is the only method of it's kind in existence for the clinical evaluation of balance disorders. The system has enjoyed a wide acceptance the world over. The system

will soon be used to assess candidates for Navy flight programs, and will become a part of the routine medical assessment of astronauts. We anticipate that future developments will assist clinicians in the selection and monitoring of patients undergoing treatment regimens and may detect persons at risk for falls in the aging.

Publications, Presentations, and Other Accomplishments:

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Neuronal Vulnerability and Informatics in Human Disease

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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 five 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. 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. 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. 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.

We are very pleased with the progress and interactions to date, and believe that the new software now coming on line will be useful to all of the Projects as well as to many neuroscientists within the HBP community.

Core and Bloom/Young Component and Morrison/Young Component

Over the past year, and the first full year of funded operations, we have concentrated on two aims: 1) software development and 2) system/hardware configurations.

Software Development

Much of the work in year 2 was devoted to developing and improving the base tools that will be used throughout all of the application programs that are proposed - NeuroZoom, NeuroBase, NeuroAtlas, and NeuroNet. Currently, all software have been developed on the Macintosh, and written in Smalltalk. Common to all four of these programs are certain fundamental classes of code that are required:

Graphical Tools - the standard tools for creating, editing, and storing primitives are done. These include basic points, lines, ovals, rectangles, open contours, closed contours, open parametric curves, and closed parametric curves. When the tools are used within the application framework, they are sensitive to the both the device coordinates of the computer, and the world coordinates of the data acquisition devices. This makes these tools much more portable to other computers and operating systems, such as PowerPC, Windows, OS/2, and UNIX. All tools were designed to be completely intuitive and to be as easy to use as any Macintosh or Windows program. The locator device (a mouse, tablet, or touchscreen) selects the desired tool from the palette and the data object is then created in the window's canvas. These data are converted immediately to real world coordinates, which in the case of NeuroZoom is on the order of 0.1 micron accuracy. Any data object created is tagged with real world information, such as the tissue structure that it represents, and can be edited in real-time. The tool classes reside in one monolithic, portable framework, called NTools, and interact with NDBObject, another frameworks to be discussed below.

Database Tools - to support document storage of data objects, database tools needed to be developed. These tools are all located in a framework called NDBObject, and provide a variety of simple database functions: indexing, keys, and persistency.

Indexing allows all data objects to be cached in a table for quick lookup from a database file. The subclass NDBIndexedPersistent supports the indexing scheme. The entire context of the object need not be resident in RAM memory. Indexing allows the application to keep only the cached table in memory, and retrieve the whole context from the database file upon demand. Many of the data objects in the applications are very large, especially when they are near the top of the document database tree. For example, NeuroZoom uses a class called a study, which contains all of the experimental data that is being analyzed or entered during an experimental session. The scope of the application includes the current study under investigation, meaning that the entire study is loaded from the database file. This improves performance on lower performance computer systems at the expense of using more RAM. However, one database file can contain an unlimited number of studies. All other studies not within the scope of the application remain in this file, and are retrieved into memory only when needed. Indexing allows the application to retrieve those studies from the database file easily and quickly.

Keys support a simple tiered relational system. Classes that resemble the functionality of relational tables can be created as subclasses from NDBKeyedPersistent. Each subclass maintains its own key lookup cache, and provides unique keys to each object of the subclass that is instantiated. The keys are

automatically stored to the database file when the object is removed from memory. NeuroZoom uses keys for all of its supported devices, which are also objects descending from NDBObject. These devices are keyed because they refer to one another as embedded objects. For example, an electronic camera (Sony CCD, Leaf Lumina, etc.) is a keyed object, but also refers to another keyed object called camera format (640x480, 512x512, 3400x2700). The camera format is also referred to by the canvases used throughout NeuroZoom to determine sizes when calculating scales and offsets. If the data in the camera offset object changes, both objects referring to it by keys are notified of the changes by relationship. In other words, changes to the one object are able to affect many other objects (one to many relationship).

Persistency is supplied by the base class NDBPersistent, from which both NDBIndexedPersistent and NDBKeyedPersistent descend. Persistency in this case is the ability to read and write oneself to and from a database file. Persistency means that the object can purge itself from RAM memory into archival storage, but still recreate itself perfectly when needed again. Without persistency, RAM limitations would hinder the performance and power of all programs. All of the document structures in NeuroZoom use these subclasses to support all forms of file I/O. The form of the persistent object is a binary representation, and designed to be read and written quickly. It is fully object oriented, such that architects of new subclasses need not know how the base classes are handling their own data. The new classes being designed only need to understand how to read and write their own data. Two functions need to be coded within the new subclass, and persistency is then automatically granted to the new subclass. The persistency mechanism operates through any streaming device, which includes networks, which will be discussed below. This means that objects that are created within an application can be easily transmitted to another computer or application on a network, such as the Internet. The format of the file containing the persistent objects will be a public API for our applications, and other languages can easily use them.

Network Tools - all of the applications have inherent network support built in. NNetwork is a full class framework designed to support networking activities. The protocols currently supported are TCP/IP in the subclass NTCPNetwork. These might be in the form of EMail, for example, where bug reports, suggestions, or internal reports can be sent to any recipient on the Internet. Likewise, mail can be received into any application from the Internet. Low level support of servers and clients are also designed. Subclasses NTCPClient and NTCPServer support the creation of any specialized server or client. Full transaction modeling based on the network is provided. Messaging, parcellation, and byte level transactions are supported. Any of the data objects described above in the database classes can be transmitted or received over the network. Because the persistency mechanism is used, architects extending these frameworks need not to know the details that are involved in these fundamental tools. A couple of functions need to be written for each new class and then the objects are network aware.

The networking classes also have support for security designed in, though they are not currently implemented. All data transmitted can be encrypted, and decrypted with a local key by the receiver automatically. Compression and decompression is also fully supported for slower network connections.

Device Tools - NeuroZoom was the first application that both the Bloom/Young and the Morrison/Young components concentrated on. It is a microscopy data acquisition and analysis program, designed to do extensive tissue mapping from light and laser microscopes. NeuroZoom is designed to be both a specialized and a general program at the same time. In other words, it can be as general as building atlases if the mapping context in the window is an image or series of images from MRI, microtome slices, etc. Or it can be specialized as controlling a microscope's motorized stage, controlling and acquiring electronic imagery, turning objectives on the nosepiece, controlling the laser parameters, etc. NeuroZoom will be discussed in more detail below. In order to support these devices, classes had to be designed to communicate with them. NDevice is the base class of the framework that does this. Within this framework, IEEE devices are fully supported. A generic driver for IEEE protocols can be used by any other object. Specific classes that use this are subclasses of NStage, such

as LudlMac1000 and ZeissMSP65. These are the two stages that will be initially supported by NeuroZoom when controlling the microscope. The next device driver to be designed will be a serial communications device, so that the same stages or others to be designed, can switch to serial communications if an IEEE board is not available in the computer. The base classes LudlMac1000 and ZeissMSP65 do not change. Only the driver is loaded for objects or those classes. The drivers have been abstracted well enough so that the interface boards can be shared with other applications running simultaneously on the same computer, or with other threads running within the same application context. Multiple IEEE devices can then be controlled simultaneously from a variety of programs.

Virtual stages have also been designed for NeuroZoom that simulate a physical stage in all respects. Moving the stage using a on-screen controller changes the mathematical offsets contained within this stage, and the NeuroZoom then reacts as if the physical stage has in fact been moved. Of course, if a live video image is currently being displayed, the image will not really move. However, a specialized subclass of this virtual can be configured in by the user as the stage device. This specialized stage uses a dialog window and the keyboard, and will ask the user to move the stage when NeuroZoom is trying to move it, and will ask for current coordinates when NeuroZoom is updating stage offset data from the stage. By using this technique and subclassing properly within the NDevice framework, NeuroZoom is able to accommodate many more users than just those with the Zeiss or Ludl stages in their laboratories.

Further plans are to create a new subclass that communicates with the Zeiss LSM410 confocal microscopy system in the Morrison lab at Mt. Sinai. This will effectively communicate with the DOS computer controlling that microscope, moving the stage and getting stage and imagery information back. Since it will be a properly designed subclass, it will appear automatically as an option in the device configuration list in NeuroZoom, and selecting it will automatically create the proper environment to use NeuroZoom on the LSM410.

Applications - Using the classes above, several applications were designed in Year 2. Two ancillary applications were made to support internal work in progress - SnapIt! and Lumina SnapIt!. Both are designed to capture serially sectioned images from some imagery sources, such as a sliding microtome sectioning whole brains. SnapIt! works with any video camera attached to a Macintosh using QuickTime. Lumina SnapIt! operates with the high resolution digital color camera from Leaf Systems, Inc. The goal was to make serial sectioning and capture easy, and to provide for some security against losing data, especially when sectioning tissue, since only a block face is presented only once for registration image capture before being sectioned off. The creation of file names are serialized with a base name that the user provides. File overwriting is thus prevented by automating this important step. Series are maintained in a database, so the process of capturing the series can be continued over several sessions. Furthermore, several regions of interest can be captured simultaneously. This is important especially with the Leaf Lumina camera because several minutes are required to grab one frame of data. If multiple sections mounted on a glass slide are being captured, such as in our project to archive the rodent brain in three planes from the Welker/Johnson collection at the Univ. of Wisconsin, each section on the glass slide can be framed during the editing phase. The acquisition of each frame is optimized by SnapIt! as part of the scanning process. Therefore, there is no significant increase in acquisition time for n number of images vs whole frame capture. This is merely a user interface issue that when done properly within the application program, saves a significant amount of time for the user. All of the Leaf Lumina code was rewritten by Mr. Michael Gertz as SCSI based calls within Smalltalk. This code is unique and will be contributed freely to other programmers.

NeuroZoom is the major application that was being developed in Year 2. It is a mapping program that works with light and laser microscopes, computerized stages, video and electronic cameras, camera lucidas, MicroBrightField's LuciVid system, and file-based images. Specialized modules in NeuroZoom support stereology mapping, which is unique because not only is stereology supported for unbiased counting and estimation of cellular densities, but it is combined with mapping such that

whole tissue sections, and even serial tissue sections, can be mapped out within one context, and analyzed with traditional morphometric tools, or applied against stereology methods. NeuroZoom works like standard Macintosh or Windows applications, asking for documents in which to store data, bringing up windows that act as the canvas in which to enter the data, saves the progress into the files, and allows the files to be portable to other computers so that the data can be shared, or imported with other applications.

Since many different devices are supported within NeuroZoom, NeuroZoom can adapt to the equipment available in different laboratories. If for example, a video board is in the computer which supports QuickTime, and an electronic camera is attached to the board, live video can be displayed in the canvas while the user is mapping. This is probably going to be the most popular way that NeuroZoom presents data to the user for mapping. As the field is mapped, the stage on the microscope, if any, is automatically or manually moved to adjacent fields. All parameters concerning the frame of movement are configurable. Many different morphometric data types are supported - points, lines, ovals, rectangles, contours, bezier curves, etc. Ms. Soraya Gonzalez coded all of the parametric cubic curve functions in Smalltalk. Any number of structural entities, such as cells, layers, boundaries, can be configured by the user for each data type. There is a complete layering mechanism, so that an unlimited number of layers can be created to assist in the organization of data. There is complete control on the presentation of the data with the use of not only the layers, but the visibility of structures, data type categories, etc.

All physical devices on the microscope that have an effect on the presentation of the images are configured by the user. These presentations are called views, and their scale factors are affected by the microscope, the camera, and the nosepiece lens objective. Once scaled, these views are available for all users of NeuroZoom, or may be transmitted to other users over a network.

When using stereology, either manual or automatic control is provided. By manual control, multisectors, which are the framed out areas in the tissue sections, can be created by mouse or keyboard. The number of dissectors within this multisector can be specified, along with the thickness, and the top and bottom Z locations. That multisector can then be mapped where NeuroZoom will move the stage (or ask the user to move the stage) to the location of the multisector, focus on the top dissector, and allow the user to use the standard mapping tools to enter in data.

By automatic control, NeuroZoom can create random multisectors anywhere within a contour. The way it works is as follows. Contours are mapped out by the user as part of the general mapping routines. These may be at any magnification. The stereology manager presents these contours to the user. One contour is selected and the number of multisectors to be generated is specified by the user. NeuroZoom will then create that many number of multisectors randomly within the area of the contour, without overlapping any of them. Each multisector in this group, conveniently called a multisector group, is then specified for the number of dissectors, the thickness, and the top and bottom Z locations. Either a singular dissector parameter's definition is stored in the multisector group object for all multisectors to use, or any individual multisector can be overridden for its own dissector parameters definition. NeuroZoom does not impose protocol, but can provide it. When the user is ready to map, the stereology manager moves the stage to the first multisector, focuses on the top dissector, and enables mapping. When the data within the dissector are statistically and significantly uniform, the next dissector is presented. When the overall data in the multisector are statistically and significantly uniform, mapping stops for this multisector, and the next multisector is presented. Finally, when the multisector group which represents this contour or area of tissue is statistically and significantly uniform, mapping for the entire group ends. Statistical significance is calculated in real-time as data are acquired, and methods such as the fractionator, the selector, and the nucleator as outlined by Gundersen et al will be used (Gundersen et al, The new stereological tools: Dissector, fractionator, nucleator, and point sampled intercepts and their use in pathological research and diagnosis., APMIS, 96:857-881, 1988).

Since NeuroZoom uses the standard database tools as outlined above, new modules can be designed into it to extract out data and present it in different ways. Work in the latter part of Year 2 will be on designing a 3D expression module that can rotate and interact three dimensionally with the data. One advantage that is inherent in NeuroZoom is that all data are intrinsically 3D. Some work has already been started by Mr. Michael Gertz in applying affine transformations to the data, and in designing the user interface tools. Mrs. Soraya Gonzalez will be continuing this work. C code has also been written to support RIB (Renderman Interface Bytestream) files that the Pixar RenderMan rendering engine wants to use. By combining this RIB support into the database classes, data can be transmitted directly to the rendering engines over the network via a server built directly in NeuroZoom. Since NeuroZoom is fully multithreaded, work by the user is not interrupted with the Pixar engine, or with our own rendering code, so that the user can continue to collect data.

System/hardware Configurations

The Bloom/Young component has organized a setup for the rapid collection of brain sections. An American Optical sliding microtome is fixed to a base with a vertical sliding head, onto which an electronic camera or a Leaf Lumina camera can be attached. A Zeiss cooling unit is attached to the microtome to keep the frozen blocks at a consistent temperature. A Macintosh 840AV with 64 MBytes of memory and 2.1 GBytes of disk space is in the same room. Using either SnapIt! and Lumina SnapIt!, registration images can be captured as the brain is being sectioned. These are stored locally on the hard disk, and then transferred to a network server for backup.

Colocalized is a Zeiss Stereoscope that can be used with either camera systems to acquire slide mounted data. This also can be collected locally by the Macintosh and then transmitted to the network server.

Both the Bloom/Young and Morrison/Young components have configured rooms for microscope control, mapping and stereology. Both have a Macintosh 840AV with 64 MBytes of memory and 2.1 GBytes of disk space connected to a Zeiss Axiophot system. The Bloom/Young system has a motorized stage built by Ludl Electronics of NY, and the Morrison/Young system has a Zeiss MSP65 and a Zeiss MCU27 motorized stage system. Supporting different stages will be a test of the level of abstraction with NeuroZoom and whether the user detects a difference in what he is currently using.

Video conferencing will once again be attempted by both groups as part of the new QuickTime Movie protocol. This protocol supports IP and thus can travel over the Internet from site to site. The protocol is also adaptive to the real-time bandwidth, and readily sizes the number of frames to update continuously to provide the best dynamic presentation between the connected sites. NeuroNet will use this technology as part of intrinsic support, distribution, and teaching, once the other major applications within our Human Brain Project consortium are completed.

The Scripps component is also supporting full WWW (WorldWide Web)and FTP (File Transfer Protocol) servers for the Human Brain Project. The WWW server reflects all other HBP grantees' WWW servers with one home page. Scripps also maintains a separate server for its own HBP activities, and this also is reflected in the main HBP WWW server. All servers have been operational since the summer of 1994.

Morrison Component

The NeuroZoom software development has progressed over the last year to the point where we will begin testing it in February (see Dr. Young's description for details on software). A major new function that has already been implemented and used throughout Year 02 is the Optical Dissector program that allows for unbiased stereological procedures to be used for neuron density calculations. This program has been used extensively for quantitative chemoarchitectonic analyses of primate neocortex. A detailed, comprehensive analysis of the density and distribution of neurofilament rich pyramidal cells in twenty eight distinct areas of primate visual cortex was completed this year and is in press in Journal of

Comparative Neurology. A similar analysis of the orbital frontal areas has been completed and is also in press in Journal of Comparative Neurology. These studies have now been extended to include quantitative analyses of subsets of GABAergic neurons distinguished by the presence of one of three calcium-binding proteins. These analyses are crucial to our overall goal of developing quantitative profiles of specific cortical regions based on chemically-specified cell classes.

Our efforts to measure intracellular levels of immunoreactive molecules on the Nonfocal microscope have also progressed in Year 02. We are now using a reliable method developed on our Zeiss LSM 410 for comparative measurements of fluorescent intensity in immunofluorescent preparations. The method has been employed successfully to measure age-related changes in glutamate receptor levels in the dentate gyrus molecular layer, the terminal zone of the perforant path. These analyses have demonstrated a significant shift in the fluorescent intensity ratio of the inner molecular layer as compared to outer molecular layer for NMDA receptors, while structural proteins do not display a similar change. This approach will be extremely valuable for several analyses of age-related, disease-linked, or experimentally induced glutamate receptor changes on the level of single neurons or neuron sets.

PLANS

The development of NeuroZoom will be completed this year, and extensive testing of the software will occur in Year 03. The programs will be modified throughout the year in response to feedback from the microscopists in the Morrison lab and at Scripps, as well as b test sites in Chicago and Australia. The quantitative analyses of signal intensity on the Nonfocal microscope will continue, and in Year 03 we will attempt to correlate such measurements with ultrastructural analyses that generate counts of synapses labeled for specific glutamate receptor subunits. The quantitative chemoarchitectonic analyses will continue, and will be expanded both in respect to cortical regions and additional labels for chemically specified systems (e.g., calcium-binding proteins, glutamate receptor subunit proteins).

Karten Component

This portion of the project consists of two major components:
1) Development of a retinal database linked to quantitative data collection
2) Digital Stereotaxic atlas: Vector based format

1) Development of a retinal database linked to quantitative data collection

The specific aims of this portion of the project is to develop a Retinal Database Linked to Quantitative Data Collection.

Graphical Oriented Database:
In contrast to our intuitive classification of graphical objects based on graphical features, most databases rely on text descriptors. Graphical images are stored, referenced and retrieved based on the users assigning text descriptors to the image. Extraction of common graphical features that would provide a basis for grouping objects is essential to any classification of cell types, faces, etc. In view of the relatively limited diversity of morphological types of cells in the retina, we will attempt to develop a graphical based system of classification of cell types. This work is being done in collaboration with Ramesh Jain of the Department of Electrical and Computer Engineering at UCSD. Professor Jain is an expert in graphical databases and has had extensive experience with various database systems. The database will initially utilize the recently developed Illustra database system. Based on those notably graphical properties of Illustra, Professor Jain hopes to develop tools for classifying the major different types of horizontal cells of the chick retina. Horizontal cells are limited in variety, with differing morphological, biochemical and physiological features, and will provide a model cell type for our studies.

Improved Data Collection and Analysis Using Confocal Laser Scanning Microscopy (CLSM):

Computerized Tools for Data Acquisition:
Collection of precise information about cell size, number, dendritic domains, transmitter, receptor and trophic factor expression is important in understanding processes of development, maturation and aging.

Quantitative Analysis of Retinal Cell Types with Confocal Microscopy:
Retinal cell types are often highly stereotyped in their three dimensional morphology, transmitter and neuropeptide content, as well as in their complex physiological features. The development of the confocal laser scanning microscope (CLSM) permits measuring the density of chemically specific cell types, their size, spacing, and precise 3D morphology of individual neurons. The retina is particularly suitable for such an analysis in view of the highly laminar organization of the cells and their dendritic arbors.

Use of NIH Image for Post-Acquisition Processing of Confocal Images:
In order to facilitate post-acquisition processing of CLSM derived images, I have prepared a manual showing how to use NIH Image, by Wayne Rasband of NIMH, for the majority of postacquisition processing. This manual is currently undergoing evaluation and modifications in response to initial testing. It will be posted on the NIMH Server (zippy.nimh.nih.gov). The manual outlines methods for quantifying data, including cell numbers, relative concentration of substances in different cell layers of retina, resectioning Z-series, generating 3D rotation series, etc. This program currently runs on both a standard Mac and on the PowerPC Mac. The program is distributed as Freeware by NIMH.

Use of CLSM to Evaluate Quantitative Changes in Photoreceptors with Age:
Using the CLSM we have begun a project of evaluating quantitative changes in specific photoreceptor cell types in the retina. The Green/Red principle cones of the quail retina show rapid reduction in number with aging. We have preliminary evidence indicating that these cells are selectively labeled with calcium binding protein (calbindin). We have now used the CLSM to quantify the numbers of calbindin positive photoreceptors in healthy young chicks. A large part of the difficulty in quantifying the loss of such cells is that they normally are most readily identifiable in transverse sections of the retina. However, in view of the geometric disposition of these cells, it is exceedingly difficult to count their density in transverse sections, and even moderate loss in cell numbers may be difficult to confirm. However, by initially collecting an extended Zseries of section from transverse series, we can now very rapidly digitally "re-section" the retina in an orthogonal plane that immediately shows a horizontal view of these photoreceptors, allowing precise counting of their number and density. Initial studies have shown that these cells form highly regular spatial arrays. Once the current series of measurements have been completed, we will begin to examine changes in this population with aging. The analysis uses methods outlined in my manual (described above) describing use of NIH-Image for post-acquisition processing of confocal images.

Computerized Microscopy and Quantitative Data Collection:
We have been collaborating with the developers of Neurolucida to develop new tools and applications based on this software. Neurolucida is a commercially available program for controlling a motorized stage, video images, and visual overlays to map the distribution of various pathways, chemical constituents, etc. This software currently runs under a DOS/Windows environment. The data files can now be readily transferred to a Mac. Within the past few months, image processing for color segmentation, cell counting, densitometric measurements, etc., have been added to the program.

Quantitative Analysis of Retinal Cell Types (Cacciatore et al., 1995)
The first of a series of quantitative studies of retinal organization has been completed and has been submitted for publication. This is an analysis of retinal areas, thickness, and cholinergic cell types in the retinae of two species of squirrel. These animals were chosen on the basis of their many similarities to the primate retina and central visual pathways, including the high number of cones, high ganglion

cell density, prominent geniculostriate and pulvinar systems, etc. We are developing a standardized format for analysis of retinae of different animals, of various cell types based on morphology, transmitter content, receptor subtypes, etc.

2) Digital Stereotaxic atlas: Vector based format
We have continued to develop a standardized format for vector based stereotaxic atlases of brains of humans as well as of various experimental animals.

In order to provide a more universal medium for data interchange, we have been exploring methods of transfer the individual files to a universal format. These files can now be utilized by a variety of programs, including Adobe Illustrator 5.5, Canvas 3.5.3, MiniCAD 5.0, and various high end graphical rendering programs (see below).

We are developing filters for export/import of these files via a DXF format (Data Exchange Format) an arbitrary CAD "standard" developed by AutoDesk, Inc. The DXF format is supported by all high end rendering programs, as well as by AutoCAD, MiniCAD, Canvas, Illustrator, etc.

a) Experimental animals - Pigeon: The pilot project for developing a standardized vector based atlas grew out of my efforts to convert the Stereotaxic Atlas of the Pigeon Brain (Karten and Hodos 1967) into digital format. An initial version of this atlas was posted on our internet server within the past year. The original version was presented in Canvas Format. It is now also posted in DXF and PICT formats. We have now completed the first stage of this project, preparation of a full series of plates in transverse and sagittal planes. We are presently in the process of converting each nucleus into a separate object, in its own individual layer. This has also provided information about the area, perimeter and coordinates of every nucleus at each level.

In addition to the vector based drawings of each section from the atlas, we have added a number of sagittal plates not previously published in the original atlas. In order to complement the line drawings, and provide the full functionality of the original paper publication of the Pigeon atlas, we have now completed digitization of all the associated Nissl stained sections of the atlas. We are experimenting with the most efficient way of distributing these files. Each Nissl stained section is stored as a single file and presently requires in excess of 1-4 MB. We are comparing the performance of various compression algorithms in retaining detailed histological information contained in each image.

An associated database file lists each structure, abbreviation, alternate name, location in stereotaxic coordinates, and functional system with which it is affiliated.

Electric Fish: The digital atlas of Apteronotus, a weakly electric fish, is still in progress, and an initial series of plates have been posted on our Internet Server.

Chick Brain: Wayne Kuenzel recently published a stereotaxic atlas of the chick brain, an animal widely used in developmental studies. Kuenzel's atlas provides a complete series of drawings in the transverse, sagittal and horizontal planes. There are no associated photographs. In collaboration with Wayne Kuenzel, we have recently begun the transfer of chick brain into digital format. This will eventually also be available on the Internet Server based at Scripps Research Foundation.

b) Human atlas - The Stensaas atlas, based on brains from the Yakovlev collection, has now been revised with regional code numbers now converted to standard anatomical nomenclature. During the coming year each nucleus will be individuated as a separate object and placed in an individual graphical layer. The original format used in this atlas was Aldus Freehand 4.0. A major limitation of this format is that there are no available filters that allow us to use this data with other graphical programs (see below). We have, thus far been limited to transferring the Aldus based files into a PICT format. This retains the vector based features, though it does not preserve the layering information. Once this has

been transferred to a format that allows us to restore the layering information, we will be able to selectively manipulate the files for optimal presentation of object oriented nuclei.

We have also developed a simple flat file database of brain structures in the human brain, using FileMaker Pro. This is a widely available, low cost, commercial database. Each structure listed in the Stensaas atlas, as well as all structures listed in Nomina Anatomica are included in this. Each nucleus is represented as a separate record, with separate fields for each nucleus, abbreviation, location in brain, functional system in which it participates, look up table of alternate names, location in various atlases, and schematic illustration of location on a few representative plates. Each record will also have a software pointer to the original graphical file containing the structure.

In addition to the individual vector based drawings, Stensaas has prepared over 5,000 color slides of representative histological fields of each region of the brain, matched to the individual regions of the brain. Storage and retrieval formats for these images is still being explored. We are currently considering either analog (addressable video disk, as in HyperBrain by Suzanne Stensaas) or digital format on CDROM. The size of the digital files is a major obstacle to rapid retrieval. The analog format is less widely available.

The vector based atlas, in multiple formats (DXF, Canvas, Freehand, PICT), as well as the associated database files, and some representative histological sections in digital format will be posted on our Internet server. Initial samples have already been posted. The preparation of the atlas and associated plates is very labor intensive, but we anticipate posting the first complete version of the atlas within the coming year.

Foote Component

The Specific Aims for this component are as follows: 1) To examine levels and distributions of mRNAs for dopamine receptor subtypes in selected regions of monkey brain following repeated psychostimulant administration. Receptor distributions in chronically treated animals will be compared with those of drug naive or acutely treated animals. 2) To examine levels and distribution patterns of dopamine receptor immunoreactivity in selected regions of monkey brain following repeated psychostimulant administration. Immunocytochemical results will help to verify that cells which show hybridization for the receptor mRNAs actually translate those mRNAs, and will give us information about the distribution of receptor in cellular processes (e.g. axons) which do not typically contain mRNA, but may well express receptor.

Tissue acquisition: We have collected aldehyde-perfused brain tissue from a total of six monkeys, a seventh is currently undergoing daily training for chair restraints and the type of handling necessary to receive infections. Pursuant to another scientific study, the animals were treated with low-dose amphetamine for two weeks according to a sensitizing protocol. We chose to administer damphetamine (0.25 mg/kg/day) in these animals because we have shown that this dose of amphetamine produces behavioral sensitization (when administered chronically) and results in considerable augmentation of dopamine release in those areas we have selected for microdialysis. In addition to microdialysis data on these animals, we have obtained good quality electroencephalographic recordings from four of these animals during their treatment and behavioral recording.

Probe selection: Morrison's laboratory has characterized riboprobes against monkey D1, D2 and D5 dopamine receptor mRNAs which he will be making available to us. In preparation for this, we have acquired supplies and equipment to carry out in situ hybridization histochemistry. Dr. John Kelsoe has generously offered a portion of his molecular biology laboratory (located four doors down the hall from us) for our use in the in situ work Kelsoe has other riboprobes which may be of considerable interest to us: these include probes directed against unique portions of the glucocorticoid receptor and the n-methyl-D-aspartate receptor (NMDAR1). A wealth of experimental data implicates these receptors in the development of sensitization to psychostimulants.

Antibody selection: We have obtained a polyclonal antiserum which was produced using the carboxyl terminus of the D2 dopamine receptor as the immunogen, and we have partially characterized its specificity in rat, human and primate. It appears to give labelling which is very similar to the known distributions of the combined populations of D2 and D3 receptors. Cross-reactivity with the D3 receptor is to be expected, since the two receptors share considerable homology in the carboxyl tail. Morrison's laboratory will help us further characterize this antibody using transfection assays and Western blots.

Several specific antibodies against the D2 and D3 receptors have been produced in Morrison's lab, using fusion proteins derived from the third intracellular loop of these receptors. Especially promising is a monoclonal antibody against the D3 receptor which has been extensively characterized. This antibody stains perikarya and dendrites in various areas of macaque cortex, yielding a pattern which is qualitatively similar in some areas to that obtained with the D2/D3 polyclonal antiserum described above. Morrison will be furnishing us with this reagent once a sufficient quantity of clone has been grown up and we will use this antibody along with our polyclonal antiserum to map the distribution of immunostainable D3 and D2 (by subtraction) receptor in drug-treated and drug naive monkeys.

Image analysis: We have acquired an additional Macintosh (Quadra 630) computer to facilitate image analysis and quantification of receptor densities as revealed by immunocytochemical or in situ methods. We will generally be using the NIH Image program (version 1.55 for floating point calculations) for offline quantification. The Quadra 630 filly supports an Ethernet connection which will facilitate file transfer from Scripps to our lab, so that large size image files can be readily downloaded at our location after they are acquired at Dr. Bloom's laboratory using his video microscopy setup. To prepare images for publications, we will also be using Adobe Photoshop software followed by digitization and color printing at Dr. Bloom's laboratory

Psychostimulant abuse is of considerable concern in the world today. Comparatively little is known about the relationship of the development of sensitization to the levels of neurotransmitter receptors (and of their mRNAs) in the nonhuman primate. Such data may be more readily generalizable to the human situation. Additionally, our studies offer the promise of correlation of neurotransmitter levels, behavior, physiology and neuroanatomy. Although an ambitious undertaking, when good data are obtained in several of these categories for the same experiment for the same animal, conclusions can be drawn with greater certainty than when relying on one type of measurement.

Jones Component

The specific aims of this project involved the digitization of Nissl-stained sections through the forebrain of macaque monkeys, of plates from the Hirai and Jones atlas of the human thalamus and of material prepared in ongoing studies of transmitter-related gene expression in the normal and diseased human thalamus. As a part of these projects, we sought to establish a user-friendly network involving scanning
and processing hardware and software that could be purchased and installed in any laboratory without expert knowledge. In the last year, we have completely scanned at high resolution the coronal series of sections through one of the monkey brains prepared for the Berman and Jones atlas. We have scanned a one in five series of sections, giving 200 images for this brain. Each image, scanned into Adobe Photoshop, requires on average 60 megabytes of disk space. Examples of these images have been made available over the internet to other members of the project and to members of other HBP projects who have sought them. The storage requirements are formidable so we have recently purchased a CD writer and are transferring all the image files to compact disks for distribution. In the coming year, we will digitize the sagittal and horizontal series of sections from two other monkey brains in the same manner as the frontal series. We have also prepared two serially sectioned human thalami, one cut frontally and the other horizontally, stained for Nissl, histochemically for cytochrome oxidase and acetyl cholinesterase, immunocytochemically for CAM II kinase, parvalbumin, calbindin and GABAA receptors, and by in situ hybridization histochemistry for GAD, CAME kinase-a and GABAA receptor

mRNAs. The frontal series has been digitized, mRNA levels quantified by optical density scanning and the description of the thalamus, based on differential patterns of gene expression in its nuclei, is being prepared for publication.
One pair of human thalami consisting of one member from a schizophrenic brain and a matched control have also been sectioned frontally and stained in the same manner as above. We have now commenced analyzing the mediodorsal nuclei with the aim of confirming if there is substantial cell loss in the nucleus in schizophrenics and if this is accompanied by major changes in gene expression for transmitter related genes.

Publications, Presentations, and Other Accomplishments:

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Environmental Constraints on Postural and Manual Control

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Task Description:

Extravehicular activity (EVA) is pivotal in supporting shuttle and station operations, including maintenance, construction and contingency tasks. This investigation will provide information to determine the most efficient and safe methods for performing EVA tasks characterized by the facilitation of robust and adaptive movement control strategies. Ground-based studies will investigate the process of adaptive behavior during posture and manual control prompted by varying environmental constraints that act to increase the potential for whole body motion relative to the workspace. Under these circumstances (apparent in performance under water, on frictionless surfaces, and in weightlessness), the provision of a support surface is recognized to qualitatively expand the capacity for effective performance. A conceptual model of the mechanism by which the placement, configuration, and dynamical characteristics of support surfaces qualitatively and quantitatively impact manual performance will be developed.

The study has identified: 1) Instrumentation for data collection in Weightless Environment Training Facility (WETF) and Precision Air Bearing Floor (PABF); 2) Issues relevant to simulator fidelity in WETF and PABF; 3) Multiple criteria for manual control in mass handling; 4) Extravehicular Activity (EVA)-relevant experimental mass-handling tasks; 5) Operational relevance and validity of investigation; and 6) Supplementary phenomenological/experiential methods.

We have reviewed and evaluated: 1) Procedures for crew training in WETF and PABF; 2) Mass handling qualities in WETF and PABF; 3) Extant EVA crew restraint systems; 4) Written documentation of EVA performance; 5) NASA plans for and conclusions about "Generic EVA"; 6) Extant EVA tools and equipment; and 7) Hypotheses and specific aims in experimental design. We have proposed and designed the fidelity enhancing modifications for the PABF, alternative restraint systems, and data presentation formats. We have developed hypotheses that deals with the effects of: 1) postural configuration on manual performance; 2) postural stability on manual performance; 3)

Orbital Replacement Unit (ORU) location on manual performance; 4) ORU location on postural control; and 5) Kinematic/ kinetic criteria for whole-body coordination. We have also gathered exploratory data and completed the experimental procedures in PABF. The outline for hypothesis testing and data analysis along with experimental design for a series of six experiments has been generated. These accomplishments are described in a technical report.

The questions we have answered are: a) the relation between the experiments and operational considerations in EVA; b) the key scientific principles behind the relation between crew member (postural) restraints and mass handling; c) methods that should be used to study the relation between postural restraints and mass handling; and d) the design and use of ground-based simulators for the development of EVA skills such as mass handling. We have determined the detailed requirements for an experimental facility that maximizes scientific and operational validity. We have identified feasible multi-DOF modifications to the PABF and to the methods for data collection and analysis that build on a broad base of scientific and operational experience and that extend the experimental domain to include essential aspects of mass handling skill. We have concluded that the essential aspects of this whole-body skill include: a) management of the tradeoff between postural stability and mobility; b) control of multiaxis postural perturbations due to noncoplanar force couples between ORU and restraints; and c) sensitivity to the postural and ORU inertia tensors with respect to the ORU trajectory and the location and orientation of restraints, EMU and ORU.

The information and conclusions from the investigation have emphasized the importance of an adaptive-control-theoretic view of the human-environment system (i.e., ORU-crew member-EMU-restraint combination). In this context, details of the mass handling task that are critical to successful completion of the EVA indicate which states of this system must be detectable and stabilizable by the crew member. An analysis of the EVA environment (including EMU and restraints) indicates which system states are passively stabilized and, thus, relieve the crew member of requirements for complete observability and controllability.

New questions that have emerged from this perspective include: (a) Do crew members tend to adopt postural configurations that minimize multiaxis postural perturbations due to mass handling? (b) Do crew members tend to adopt postural configurations that promote manual force vectors that, in turn, minimize cross-coupled reaction forces from the ORU? (c) Do crew members learn the inertia tensor for the ORU and attempt to apply forces along the principal axes or planes of inertia? (d) Do crew members engage in exploratory behavior that identifies tensorial properties of the ORU and EMU? (e) To what extent do different restraint systems help or hinder a-d above? (f) To what extent do different tasks emphasize or downplay a-d above? (g) To what extent do different simulators promote or discourage a-d above and do such differences influence transfer of training between simulators?

The development of more focused hypotheses about mass handling in EVA has resulted in increased emphasis on performance in an EMU at the expense of shirt-sleeve conditions. The latter have been dropped from the experimental plan. Increased understanding of the capabilities, limitations, and needs for EVA during construction of ISSA has led to an increased emphasis on operational relevance and external validity of the investigation. Supplementary phenomenological measures have been added so that expert opinion can continue to play an important role in the investigation. Finally, the recumbent EMU orientation on the PABF will be emphasized because this is the best facility to test the emerging question concerning subtle coordination between postural and manual control.

Performing visual-manual tasks while sitting, kneeling, or standing is so common that it is taken for granted until there is an obvious problem. Problems can be created by environmental constraints (e.g., workspace design/accessibility, vibration, weightlessness, visibility/illumination), musculoskeletal constraints (e.g., pain, weakness, paralysis, or other neurological disorders) or sensory constraints (e.g., poor vision, dizziness, disorientation, numbness, proprioceptive insensitivity or other neurological disorders). Problematic constraints are encountered on Earth and in space; and they can lead to unacceptable levels of performance, fatigue, and injury. Such problems can be alleviated through the

design of work environments that promote coordination between postural control and manual control or at least 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.

This research, however, does not specifically seek to develop new therapeutics or protocols for Earth but such conclusions and applications will be implicit in whatever understanding emerges about problems in coordination of postural control and manual control. Methods for alleviating problems will be suggested wherever possible.

This research addresses the coordination of postural control and manual control. The skill of coordinating such nested body systems is relevant to most of the physical tasks in which humans engage. Moreover, this skill is necessitated by upright posture and, arguably, is the raison d'etre for uprightness. The adaptive-control-theoretic approach to coordination of nested systems in this research will provide 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. 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.

The results from this research could have an impact on the "common man" to the extent that it leads to or suggests therapies, protocols or assistive technologies that can alleviate problems imposed on the general skill of coordinating postural and manual control. One of the investigators is actively involved in other research that seeks to identify assistive technology needs of individuals with disabilities. Such outside activities should promote connections between this NASA research and potential non-NASA applications. This research does not specifically seek to develop new technologies but such applications will be implicit in whatever understanding emerges about problems in coordination of postural control and manual control. Technological methods for alleviating problems will be suggested wherever possible.

Publications, Presentations, and Other Accomplishments:

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The Role of Vestibular Information in Adaptive Modification of Eye, Head, and Hand Coordination

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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 alteration 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 operation; 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 our first two studies provide insight into whether vestibular information can be used to spatially code goal-directed manual localization of remembered targets in darkness. We compared subject's ability to point accurately at a fixed target displayed on a plain background versus at a fixed target displayed on a featured background. We then compared subject's pointing accuracy following whole-body rotation in the light versus in the dark. We presented the results of the second study at the Society for Neuroscience Annual Meeting in San Diego, CA in November, 1995. 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, TX in April, 1995. A paper titled "A system for the accurate measurement of pointing responses" was accepted for publication in the

Journal of Neuroscience Methods. This paper presents the system used in our lab for measuring the direction of pointing responses.

Through this study we have answered the questions: Will differences in visual context of target display affect the subjects pointing accuracy? Can vestibular information alone be used to spatially code manual pointing responses?

This allowed us to find that in order to proceed with the adaptation phase of our study the following questions must be answered : 1) What is the minimum exposure time to minifying lenses needed in order to reduce the vestibulo-ocular reflex (VOR) gain by 20%? 2) What is the readaptation time after exposure to minifying lenses?

The results of the study serve as the foundation for our on-going investigation aimed at determining how the oculomotor and manual systems share information and how this information may be susceptible to common adaptive distortion following exposure to conflicting visual-vestibular stimuli.

This research seeks to understand a disease or malady that affects humans on Earth and/or in space. 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 the 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 conditions 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 hypothesis to be made concerning how this interaction is modified following exposure to microgravity conditions.

The development of unique research protocols 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 in clinical conditions.

Publications, Presentations, and Other Accomplishments:

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Biochemical Adaptations of Anti-Gravity Muscle Fibers to Disuse Atrophy

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Funding:

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Task Description:

The direction of the task remains the same. Minor changes have been made to accommodate opportunities for collaboration and because of results from data collection. One original aim 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 a-actin promoter, and still intend to. 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. Secondly, 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.

Our task can be presented by discussing the numerous projects.

A. Cytochrome c. Even though immobilization of a skeletal muscle in a lengthened position prevents muscle atrophy, it is unknown whether this treatment would prevent the decrease in mitochondrial biogenesis. We found that regardless of muscle length in immobilized limbs, the mRNA of a marker for mitochondrial biogenesis, cytochrome c, decreased. Cytochrome c mRNA per mg muscle was 62% and 72% *less* one week after fixation of the soleus muscle in a shortened and lengthened position, respectively, than age-matched controls. Cytochrome c mRNA was 36% and 32% *less* in the tibialis anterior muscle fixed for one week in the shortened and lengthened positions, respectively, as compared to age matched controls. Recently, we identified a novel RNA-protein interaction in the 3'-untranslated region of cytochrome c mRNA which decreases in chronically stimulated rat skeletal muscle. The RNA-protein interaction in the 3'-untranslated region of cytochrome c mRNA in soleus and tibialis anterior muscles was unaffected by fixation in either shortened or lengthened positions. We conclude that the decrease in cytochrome c mRNA in skeletal muscle is a dissociated event from muscle atrophy.

B. Mice as a model for hindlimb non-weight bearing. Except for Dr. Steffen's publication, *Aviat. Space Env. Med.* 55:612–616, 1984, we are unaware of reports that employed mice for hindlimb non-weight bearing. In order to perform experiments with transgenic mice, an apparatus for hindlimb non weight bearing was created. We had heard that mice were able to twist their bodies and climb the connection from their tail to an overhead support and to sit on the support during non-weight bearing. We developed a barrier to prevent this and found the following data. Mice (ICR strain) who were hindlimb non-weight bearing for 1 week had decreases of 6% in body weight, 24% in soleus wet weight, 14% in tibialis anterior wet weight, and 11% in lateral gastrocnemius wet weight. These successful results permitted us to employ transgenic mice in non-weight bearing experiments.

C. Slow troponin I. Slow troponin I mRNA in the soleus muscles of mice who were hindlimb non-weight bearing for 1 week were decreased to non detectable levels. Transgenic mice carrying -4200 to +12 bp of the human slow troponin I promoter driving the reporter gene, chloramphenicol acetyltranferase (CAT), were hindlimb non-weight bearing for 1 week. We are presently analyzing CAT activities in these animals.

D. Insulin-like growth factor I (IGF-I). Transgenic mice carrying the skeletal a-actin promoter driving the expression of IGF-I underwent non weight bearing for 2 weeks. Whereas soleus muscles of control mice atrophied 17%, soleus muscles having an overexpression of IGF-I in their muscles atrophied only 8%. As the number of observations per group was only 4, we will be adding more muscles.

E. Gene Medicine. We are also collaborating with GeneMedicine, at their expense, on a gene therapy project to prevent muscle atrophy. Marginal success has been obtained. I am under a confidentiality agreement with GeneMedicine and am unable to give more details other than to indicate that this work is complementing the experiment with transgenic mice that overexpress IGF-I in skeletal muscle. We would not have been able to undertake this collaboration with GeneMedicine if we had not trained personnel from this NASA proposal who are able to donate their time.

F. Crude nuclear extracts. Dr. Carson in Dr. Booth's laboratory has been successful in scaling down a procedure to isolate nuclei from 300 mg of skeletal muscle. Crude nuclear extracts from control and non-weight bearing muscles are being used to perform gel mobility shift assays. We have used this procedure and observed that the -192 promoter of the mouse myosin heavy chain IIb gene had no alteration in DNA-protein interaction because of non-weight bearing. We have performed pilot experiments with radiolabeled oligmers of the serum response and MCAT elements of the mouse skeletal a-actin gene with crude nuclear extracts from control and non-weight bearing skeletal muscles.

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, which will increase as more Americans reach the age of frailty.

Publications, Presentations, and Other Accomplishments:

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Physiological Effects of Decompression-Induced Venous Bubbles

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Initial Funding Date: 6/95
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FY 1995 Funding: $93,374
Students Funded Under Research: 2

Task Description:

Venous air bubbles result from moderate (≥ 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 illness. Risk is estimated as high as 20% based on extensive ground-based studies. Although the operational incidence of qualitative symptoms of decompression illness is remarkably low, the incidence of quantitative biochemical markers may be much greater 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 animal models. The results of these studies will enable better assessment of the physiological risk of decompression illness and begin to establish utility of operational monitors using body fluids such as blood or urine for quantitative evaluation.

The efforts accomplished thus far on the task include the following: 1) Incorporation of reproducible assay techniques (enzyme immunoassay) for the quantification of eicosanoids (thromboxane B2, 11-dehydro thromboxane B2, leukotriene E4) from experimental animal models experiencing venous air embolism (VAE) subsequent to infusion or decompression. 2) Incorporation (modification) of reproducible assay techniques for the quantification of myeloperoxidase from neutrophils, bronchoalveolar lavage and lung tissue samples. 3) Identification of pertinent eicosanoids that are effective markers in the evaluation of decompression illness resulting from VAE. 4) Completion of venous bubble infusion experiments that will be correlated with decompression-induced VAE changes.

The questions answered so far include the critical methodological concerns and identification of appropriate eicosanoids for evaluation of decompression illness. The sample sources and techniques

included not only those for blood and tissue, but also required modification for bronchoalveolar lavage and urine.

The future work on this task involves the completion of the decompression exposures so as to correlate the VAE data with the decompression-induced VAE data, in terms of a) eicosanoid production, b) lung injury and c) expression of adhesion glycoprotein complex.

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.

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.

Publications, Presentations, and Other Accomplishments:

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Robinson, R.R., M.F. Doursout, M.R. Powell, T. Little, and B.D. Butler. "Cardio-pulmonary effects under conditions of simulated microgravity and decompression in the rat." Aviat. Space Environ. Med., (Abstract) 65, 469 (1994).

The Biomechanics of Exercise Countermeasures

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FY 1995 Funding: $96,877
Students Funded Under Research: 6

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 1g.

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.

A major component of the Year 1 research activities has been the design of a tethering harness which will enable us to conduct future research at 1g equivalent loads. Previously, the largest loads that had been applied to subjects while they were exercising in the device was 60% of 1g body weight. The additional loads have been accomplished by a redesign of the Gravity Replacement System component of the Penn State Zero-gravity Simulator (PSZS). Subjects had previously complained that, even at 60% load, the waist-belt gravity replacement loading design was not a comfortable system and might, therefore, reduce compliance with an exercise countermeasure program. This problem has been addressed through design of a gravity replacement harness worn over the shoulders which bears four shoulder and four waist-level load attachment sites symmetrically distributed to anterior/posterior and left/right locations.

Two experiments were performed to accomplish this task. Eight volunteers served as subjects in each study. The objective of the first experiment was to quantify the ground reaction forces, tensions in the tethering springs, and subjective ratings of comfort from subjects wearing one of four restraint harness designs with a 60% of body weight load from the spring tensions while walking or running in the Penn State zero-gravity simulator. The four spring conditions tested were: "no springs", "shoulder springs", "waist springs" and "both (waist and shoulder) springs". Tethering subjects by the waist and

shoulders, i.e. the "both springs" condition, was the least uncomfortable condition for the subjects. The average tensions in the tethering springs were similar in all the conditions, although there was significantly more tension fluctuation in the "both springs" condition. The maximum ground reaction forces were highest in the "shoulder springs" condition.

The objective of the second phase of the experiment was to measure the ground reaction forces, tensions in the tethering springs, and subjective ratings of comfort from subjects enduring a 60%, 80%, or 100% load from either the "waist and shoulder" springs harness or the "shoulder springs" harness. When comparing the harness designs at the full body weight load, no differences in comfort level were determined. As in the first experiment, the average spring tensions were similar in both conditions, although there was significantly more tension fluctuation in the "both springs" condition. The maximum ground reaction forces were highest in the "shoulder springs" condition.

In the next three months, we plan to complete a further series of experiments designed to assess ground reaction forces, muscular activations, and limb segment positions of the lower extremity during overground locomotion, treadmill locomotion, and fully-loaded zero gravity simulated locomotion in order to gain insight into the effectiveness of the current exercise regimen used by NASA as a countermeasure against muscular atrophy and bone demineralization which occur in weightlessness.

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.

Publications, Presentations, and Other Accomplishments:

Cavanagh, P.R. "Perspectives on operational musculoskeletal research in space: physiology and biomechanics (Abstract)." May 1994 Annual Meeting of the Aerospace Medical Association, San Antonio, TX, 1994.

Davis, B.L., Cavanagh, P.R., and Perry, J.E. "Locomotion in a rotating space station: a synthesis of new data with established concepts." Gait & Posture, vol. 2, 157-165 (1994).

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Gravity in Human Oculomotor Control, Perception & Action

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Funding:

Project Identification: 199-16-12-40

Solicitation: 93-OLMSA-07

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Students Funded Under Research: 11

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.

The Two Axis Human Rotation Device that was formerly used by Professor Sheldon Ebenholtz at the SUNY College of Optometry was donated and shipped to NASA-Ames Research Center as an outright gift to NASA. The device, designed to position human subjects at precisely determined orientations about the roll and pitch body axes, has been reassembled, and is currently located in Dr. Cohen's laboratory in Building N-239 at Ames.

A new miniature goggle-mounted ISCAN infrared video camera has been ordered. This new camera weighs less than 0.5 ounces, and will allow us to obtain more precise measures of oculomotor control in various acceleration environments.

Dr. Sheldon Ebenholtz, Distinguished Professor of Vision Science at SUNY College of Optometry in New York City, worked with Dr. Cohen and his staff this summer while serving on an IPA at Ames Research Center. Dr. Alan Hein, Professor of Brain and Cognitive Science at MIT, worked with Dr. Cohen during July as an ASEE summer fellow. 'Drs. Hein and Cohen designed new experiments and agreed on the basis for preparing manuscripts that will be submitted for publication. Dr. Larry Guzy, Professor of Psychology at SUNY, Oneonta, was an ASEE summer fellow in Dr. Cohen's laboratory this summer. Dr. Guzy prepared experimental protocols and collected pilot data for a study that examined the simultaneous effects of body and background orientation on perceived eye level. Ms. Jeannine Mealey and Ms. Muriel Cummings have been hired by Biotech Services to support these research efforts at Ames.

On March 17, 1995, we completed data collection for research protocol HR122 at the 20-G Human Centrifuge Facility. This study was a major investigation that involved several hundred data runs with sixteen individual subjects in which measures of perceived target elevation and eye elevation (using infrared video oculography) were obtained at 1.0, 1.5, and 2.0 Gz. These data have been reduced and processed, and are currently being analyzed. Pilot studies on human perception of the zenith were conducted by Dr. Arnold Stoper at CSU, Hayward during this past year. Dr. Stoper is continuing these studies, and is currently working with Dr. Cohen to develop a quantitative model that will integrate these results with our previous findings.

Research Protocol HRII-071 was run with twenty-eight subjects during the summer and the autumn of 1995. The overall study examined the reaction times of upright and supine subjects who discriminated between cartoon representations of happy and sad faces that were presented in their normal erect orientations and in orientations that were rotated on the subjects' retinae at various angles from erect. The role of gravity in processing these visual inputs was examined by comparing reaction times when the subjects were erect with those obtained when the subjects were supine. Our data from both erect and supine subjects revealed increased reaction times as the images deviated from their normal erect orientations on the retinae. There were no significant differences between erect and supine conditions, indicating that this effect is independent of the direction of gravity, and that it depends, rather, on retinal orientation. Additional studies along these lines are in process.

Data collection on ten subjects for Protocol HRII-072 was completed in September of 1995 This experiment was designed to replicate and extend the findings of the previous study by Cohen & Guzy (1995). We used an ISCAN infrared video camera system to assess the relationship between eye position and target placement when a subject looked into a pitchbox, and attempted to place a target at the apparent horizon. As in the previous study by Cohen and Guzy (1995), this research systematically manipulated both the pitch orientation of the background (optical pitch) and the pitch orientation of the subject's body to evaluate the relationship between visual and gravitationally-referenced stimuli.

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 of this information is optimized.

Publications, Presentations, and Other Accomplishments:

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Hein, A., and Cohen, M.M. "Gravity contributes to the perception of visual patterns." Eastern Psychological Association, Boston, MA, April, 1994.

Effects of Acute Intense Exercise and Microgravity on Mechanisms Associated with Blood Pressure Regulation in Humans

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Students Funded Under Research: 4

Task Description:

Reductions in plasma volume (hypovolemia relative to 1G) 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.

First year funding for this task was received during the third quarter of FY 95. Since that time, we have completed the set up of testing facilities, which includes the bed rest facility, lower body negative pressure chamber, clinical lab, and data acquisition system. Additionally, we have just completed Phase I testing which included exposure of seven subjects to 16 days of 6° head-down tilt. With these data, we have been able to describe the effects of human exposure to a ground-based analog of microgravity on baseline autonomic activity, catecholamine metabolism, peripheral thermoregulatory mechanisms, aortic baroreceptor responsiveness, cardiac and peripheral adrenergic receptor responsiveness, characteristics of lower back pain, and how acute exercise may be used to ameliorate these effects. In the second year, we plan to complete experiments that will provide new insight into the potential use of acute intense exercise to reverse detrimental effects of adaptation to microgravity on mechanisms associated with blood pressure regulation and orthostatic tolerance.

Results from our experiments should provide new understanding of mechanisms underlying the clinical condition of orthostatic hypotension, from patients who are restricted to prolonged bed rest 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.

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Evaluation of the Hemodynamic Mechanism Underlying Cardiovascular Adaptation in a Chronically Instrumented Rhesus Model During Simulated Microgravity

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Funding:

Project Identification: 199-14-17-07

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Joint Participation: DoD (USAF)

Solicitation:

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Students Funded Under Research: 4

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 bed rest 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, 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: 1) that there is an increase in cardiac compliance associated with exposure to microgravity and/or 2) 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 the experimental design, engineering build-up, and veterinary preparations during the first year. The engineering research section has completed biosensor studies in which Triton ART2 flow probes and Millar 3 Fr double- and single-sensor micromanometer pressure transducers for our chronically-instrumented primate model were tested, evaluated, and selected. Seven publications and two patents were submitted from this work. Electronics instrumentation and signal conditioning system for data acquisition of hemodynamic measurements were fabricated, tested, validated, and implemented. Electromechanical support hardware was designed, fabricated, and implemented. The end product of these efforts resulted in one-of-a-kind support hardware that include head-down-tilt tables, subject instrumentation protective jackets and shields, feeding stations, and lower body positive and negative pressure apparatus (chamber, skirt, and control system). Finally, a data acquisition system using National Instruments A/D board and LabView software was configured for real-time analog-to-digital conversion of the hemodynamic parameters measured. These data are then analyzed using custom software written in MatLab. This data acquisition and analysis system enables us to analyze approximately 15 hours worth of data per subject (30,000 beats), calculating 18 cardiovascular parameters on a beat-to-beat basis within hours of each procedure. The accuracy and repeatability of this approach will ensure high reliability of results.

Selection of test subjects of appropriate size and health was completed and subject jackets and harnesses used during training and study procedures were designed, fabricated, and implemented. Training procedures enabling test subjects to adapt to the head-down tilt table in the 10 degree head-down, supine, and 80 degree head-up positions prior to study participation and methodology for the use of transesophageal echocardiography (TEE) on the rhesus monkeys were finalized. Surgical methodologies designed to reliably obtain intracardiac pressures and prevent transcutaneous exit site deterioration in chronically instrumented subjects were refined.

We have successfully completed testing of three subjects, have analyzed their results, and remain on schedule to complete testing of remaining seven subjects by May 1996. Preliminary review of hemodynamic data from these subjects are consistent with space flight and ground-based observations.

In reviewing preliminary data, we have developed additional questions that may impact this and future studies. We selected ketamine as a sedative during TEE and lower body positive and negative pressure procedures. A review of literature indicated that it does not alter cardiovascular and/or baroreflex function. We have observed, however, that there is an acute response to ketamine by either bolus injection or steady infusion that lowers aortic and left ventricular pressure, aortic flow, and alters systemic compliance and resistance for as long as approximately three minutes. Following this three minute time period, hemodynamic parameters achieve a constant, steady-state level while the subject remains sedated. In addition, we have observed elevations in aortic, left ventricular, and right atrial pressures and aortic flow following insertion of TEE probe. It remains uncertain, however, whether TEE insertion alters baroreflex response. We have designed an experiment to determine the effects of ketamine and TEE insertion on cardiovascular function and baroreflex responsiveness, and a collateral study will be conducted. Results of this study are of paramount importance to our head-down tilt study as well as future studies in which the use of ketamine is proposed.

In the third year of this project we will be focusing on completing analysis and interpretation of these data in order to answer the primary questions. Results from these experiments should provide new understanding of mechanisms underlying the regulation of plasma volume and cardiac filling pressure (CVP) during conditions of physical deconditioning or restricted bed rest. 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.

Publications, Presentations, and Other Accomplishments:

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Load-Induced Cardiac Hypertrophy

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Funding:

Project Identification: 199-08-17-72/P01HL48788 Solicitation: RFA

Initial Funding Date: 8/93 Expiration: 7/98

FY 1995 Funding: $74,996 Students Funded Under Research: 0

Joint 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 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.

The first specific aim of this project is to 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 published in the *American Journal of Physiology* entitled "Growth Effects of Electrically Stimulated Contraction on Adult Feline Cardiocytes in Primary Culture." Future plans include continuing to develop a model in which cells will be cultured in a protein matrix. This protein matrix will be made either of agarose or collagen type I. There are a number of advantages and disadvantages to each protein. Therefore, we are performing experiments with both simultaneously. Preliminary studies indicate that long-term (7-14 days) culture in either of these matrixes will preserve phenotype and function. The second specific aim of this project is to 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 model outlined under Specific Aim #1 combined with electrical stimulation, cardiac muscle cell mass increased over a seven day period of time in part because there was a significant increase in protein synthesis rate. These results are detailed in a manuscript published in the *American Journal of Physiology* entitled, "Growth Effects of Electrically Stimulated Contraction on Adult Feline Cardiocytes in Primary Culture." Future plans include similar studies with cells embedded in a protein matrix. In addition to electrical stimulation, we will alter load by changing the concentration of protein in the matrix and thus alter its stiffness. We have quantified the constitutive properties of agarose gels in a range of agarose from 1-10%. We are in the process of doing the same quantitative studies for the collagen gels from 1-10%. Preliminary data suggest there is a direct relation between protein concentration in the matrix (and thus matrix stiffness) and protein syntheses rate. As gel stiffness increases, the load on the cardiocyte increases during electrical stimulation.

The fourth specific aim of this project is to 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 1A receptor blockade with Losartin did not inhibit the growth effect of electrical stimulation. Thus, load independent of neurohumoral activation, can regulate growth, cardiac and cardiocyte mass. These data will be published in the *American Journal of Physiology* in a manuscript entitled, "Comparative Effects of Contraction and Angiotensin II on Growth of Adult Feline Cardiocytes in Primary Cultures." Studies examining the specific aims #3 and #5 are currently in progress.

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, cardioctye 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.

Publications, Presentations, and Other Accomplishments:

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Blood Volume Regulation in Primates During Space Flight

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Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95
Expiration: 2/97

FY 1995 Funding: $178,290
Students Funded Under Research: 1

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.

Four Rhesus monkeys, two males and two females, were instrumented following a 45-day quarantine period, with aortic and right atrial catheters and an aortic flow probe. After recovery, the animals were conditioned to sit in a primate chair until they tolerated it well for a period of six hours. After the animals were fully adjusted to the chairs, they were introduced into the space flight simulation studies. These consisted of water immersion to the level of mid-chest for a period of 72 hours. Since these studies had not been done, it was necessary to first obtain control data for the normal responses of the animals to this period of immersion.

Animals were tranquilized with ketamine and then a catheter was inserted into the bladder for urine collection. The animals were then put into a watertight jacket and placed into the primate chair. The jacket covered the lower body up to the level of the mid-chest. When the animals were fully recovered from the ketamine, control pre-immersion data were obtained for two hours. This consisted of normal cardiovascular data, blood levels of ANF, and the determination of the reflex for each animal. At that time, the animal was placed into the tank. The baroreflex was determined each day and just prior to de-immersion. Immediately upon de-immersion, the baroreflex was again determined. Blood samples were taken daily for ANF, blood electrolytes, creatinine and CBC. Urine was collected continuously and analyzed for sodium, potassium and creatinine. During the experiment, catheters were kept patent by infusing 3 ml/hr/catheter lactated Ringers solution. The animals were also hydrated with 45/hr of lactated Ringers solution.

To date, 72-hour immersion experiments have been completed on the four animals. Problems involved include the development of a watertight suit that was flexible enough to allow the water to produce its pressure against the body. Several experiments were prematurely terminated because of a leaky suit. There have also been some complications with the instrumentation. Presently, the four monkeys are healthy and functioning well.

When the animals are placed into the immersion tank, there is an increase in blood pressure (5-10 mmHg), cardiac output (25-50%), and right atrial pressure (6-8 mmHg). The heart rate decreases by about 10-20 bpm. The baroreflex curve shifted upward and to the right. There was an increase in ANF and in urine volume. However, the animals were always in positive water balance. The monkeys became tachycardiac when removed from the tank. Blood pressure decreased slightly in some experiments.

We feel that it is necessary to do the studies without hydrating the animals. Proper, continuous hydration may prove to be the countermeasure for the cardiovascular deconditioning observed with space flight. The initial hypothesis was to prevent or reduce the hypovolemia with chronic salt loading. It may be that the hydration studies performed accomplished that, accounting for the lack of a post-immersion hypotension. This should be answered with the "self-hydration" studies that are ongoing. After these are completed, we will begin examining responses of the animals to a volume expansion before and during immersion in order to see if there is a difference in this control system. The control volume expansions will be conducted the day prior to the immersion studies. We will then chronically salt-load the animals and repeat the baroreflex studies and the volume control studies.

Posture Load-Induced Bone Maintenance: A New Hypothesis

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Initial Funding Date: 1/93
Expiration: 1/96
FY 1995 Funding: $80,000
Students Funded Under Research: 1

Task Description:

One of the most puzzling effects of low gravity on the human skeleton is the continuous loss of bone mineral that occurs despite intensive exercise regimens. We offer a provocative new hypothesis, based on a recent theory proposed by Weinbaum, Cowin, and Yu, to explain why conventional exercise regimens do not appear to be effective, and we propose alternative mechanisms for stimulating bone growth in space.

It is widely believed that the principal stimulation for bone maintenance on earth is the low frequency (1-2 Hz) bone strain (1000-3000 microstrain) that is typically experienced during locomotion. It is these strains that exercise regimens in space attempt to replicate. A new hypothesis model was proposed by Weinbaum, Cowin, and Yu for the mechanosensory mechanism by which bone cells detect strains and communicate them to the osteoblasts that line the surfaces of bone and produce new bone mass. The mathematical model to explore this hypothesis predicts that the loading of bone produces fluid shear stresses on the membranes of the osteocytic processes in the lacunar canalicular system that are of the the same order (10-20 dynes/cm^2) as the shear stresses on the endothelium in the vascular system. Frangos and coworkers have demonstrated that CAMP, IP3, and PGE2 production is elevated in osteoblasts subjected to such shear stresses. The Weinbaum model proposes, in addition, that this type of excitation is of special significance for bone tissue, since all osteocytes are linked with neighboring osteocytes via gap processes and are similarly connected with bone lining cells and osteoblasts. The opening and closing of these communicating junctions have been demonstrated in many other cells to be regulated by intracellular Ca^{2+} ions. This leads to a change in intracellular potential between cells, the electrical excitation signal.

A fascinating discovery just reported by McLeod et al. is that, in addition to the strains due to locomotion, there are low amplitude (100-250 microstrain) high frequency (15-25 Hz) bone strains, possibly associated with muscular contractions due to posture. These strains have heretofore been ignored as a stimulus for bone growth because of their low amplitude. Preliminary results from our theory predict, rather surprisingly, that the fluid shear stresses on the membranes of the osteocytic processes due to these low amplitude high frequency strain components can be 2 to 3 times as large as the shear stresses due to locomotion and thus might be the principal stimulus for bone growth.

We have extended the theoretical model to predict the frequency response to loading of the fluid shear stresses on the surface membranes of osteocytic processes in a trabecular element. We have also extended the theory to predict the frequency response to loading of the fluid shear stresses on the surface membranes of osteocytic processes in an individual osteon. This problem differs from the one above because of very different drainage conditions and a cylindrical versus rectangular geometry.

Task #3: Task 3, which has not yet been accomplished, is to extend the theoretical model to predict the frequency response to loading of the fluid shear stresses on the surface membranes of osteocytic processes in a pie-shaped section of cortical bone. This will include the responses of lamellar bone on the endosteal and periosteal surfaces and in a central region dense in osteons, each bone matrix section being covered by a layer of cells. This is a very difficult problem as the interaction of the fluid in the vascular porosity associated with the Volkmann canals and the Haversian lumens (order 10 um) and that of the lacunar-canalicular porosity associated with the fluid space surrounding the osteocytes (order 0.1 um) are involved; these two systems are separated by the osteal layer of cells. In addition, we have determined the magnitude and frequency of oscillation of the fluid shear stress conditions on a stationary confluent osteoblast monolayer due to the oscillatory channel flow.

The specific objective of this research is to uncover the mechanism by which small strain magnitudes at higher frequencies are capable of maintaining bone as well as larger strains at lower frequencies. It is widely believed that the principal stimulation for bone maintenance on earth is the low frequency (1-2 Hz) bone strain (1000-3000 microstrain) that is typically experienced during locomotion.

These research results will contribute to the understanding of basic biological processes of bone maintenance in humans, and will therefore be applicable to the design of strategies for the prevention of osteoporosis and strategies for enhancing the long term stability of structural bone implants like artificial hips and knees.

A new hypothesis and model were proposed by the PIs for the mechanosensory mechanism by which bone cells detect strains and communicate them to the osteoblasts that line the surfaces of bone and produce new bone mass. The model to explore this hypothesis predicts that the loading of bone produces fluid shear stresses on the membranes of the osteocytic processes in the lacunar canalicular system that are of the order (10-20 dynes/cm^2). Co-investigator Frangos and co-workers have demonstrated that osteoblasts subjected to such shear stresses respond biochemically. Our model proposes, in addition, that this type of excitation is of special significance for bone tissue since all osteocytes are linked with neighboring osteocytes via gap processes and are similarly connected with bone lining cells and osteoblasts. Thus cells may transmit an electrical excitation signal to one another by changes in intracellular potential between cells.

It has been reported that, in addition to the strains due to locomotion, there are low amplitude (100-250 microstrain) high frequency (15-25 Hz) bone strains, possibly associated with muscular contractions due to posture. These strains have heretofore been ignored as a stimulus for bone growth because of their low amplitude. Preliminary results from our theory predict, rather surprisingly, that the fluid shear stresses on the membranes of the osteocytic processes due to these low amplitude high frequency strain components can be 2 to 3 times as large as the shear stresses due to locomotion and thus might be the principal stimulus for bone growth. If these findings are correct, the important activity that is lost in space is not the loading due to locomotion but the loading due to muscular contractions to maintain posture.

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Autogenic Feedback Training as a Preventive Method for Orthostatic Intolerance

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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.

All research efforts for this study were concentrated on development of a PC based system which could be used to generate displays and calculate indices of cardiovascular dynamics needed to conduct blood pressure training. In a preliminary pilot study, it was determined that measures of cardiovascular dynamics could be calculated and displayed in real-time using a PC. These included non-invasive measures of cardiac output, stroke volume, Heather index, blood pressure, total peripheral resistance and central volume. Further, it was determined that these measures change reliably when test participants are subjected to a lower body negative pressure (LBNP) and should be included among the parameters used for "feedback" in training control of orthostatic intolerance.

Next it was necessary to determine the best way to provide these new feedback signals to subjects in addition to the existing 12 parameters used in Autogenic-Feedback Training Experiment. We made the decision, with concurrence of management, that it was not cost effective to replace/modify these analog displays because the hardware was more than 15 years old and had become functionally obsolete. The functions of digital panel meters, signal conditioners, signal processors, auditory tone controls and analog waveforms displayed on wide-screen oscilloscopes were converted to software.

The PC training configuration includes a 586 processor with a 16 bit analog-to-digital converter and a multiple video display adapter (4 monitors) that utilizes software applications which we developed. The user-interactive software can directly measure and display physiological responses in real-time. Further, real-time calculations enable non-invasive measures of cardiovascular dynamics (e.g., total peripheral resistance).

This hardware/software conversion makes operation of experiment protocols much easier, less prone to operator errors and more flexible. The new configuration can also be readily duplicated for purposes of technology transfer of our research application to remote sites. We have applied for a patent for this technology.

This hardware and software development task has been completed. We are now in the process of conducting tests with human subjects. We are looking forward to completing the proposed research in the coming fiscal year. In addition, we plan to initiate one or more Space Act Agreements with university medical schools to test AFTE as a treatment for patients suffering from nausea and hypotension.

Our research group produced two CD-ROMs (through the NASA Ames Research Center, Life Sciences Data Archival Project), containing approximately 500 hours of human biomedical data in space obtained during two shuttle missions (SL-J and SL-3). The CDs contain 7 channels of raw data (e.g., electrocardiogram) sampled at 100 Hz, one-minute means of processed data (e.g., heart rate, respiration rate), and graphic displays of mission data (ten-minute means) which show physiological changes occurring across days in space. These CDs preserve astronaut privacy and are available upon request (refer to the Autogenic-Feedback Training Experiment Data). These data will eventually be placed on the internet for access by the general and scientific community.

AFT is a method for training human subjects to voluntarily control several of their own physiological responses within a 6 hr 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 include autogenic feedback training exercise as a potential treatment for chronic intestinal pseudo-obstruction syndrome and inclusion of AFTE methods in pilot training protocols for military, private and commercial crews.

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Microvascular Changes During Microgravity

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Task Description:

The long-range objectives of our research are to understand the mechanisms by which microcirculatory structure is regulated and evaluate the impact of structural changes on microcirculatory function. Changes induced by simulated microgravity provide an opportunity to extend our observations from hypertension models into physiological situations in which similar microcirculatory alterations occur. This research will strengthen our existing Program and provide important new data focusing on the microcirculation. Some of the most striking changes that develop in certain organs during exposure to microgravity and subsequent reloading occur in the microcirculation. Studies by our group indicate that chronic head-up tilt results in vascular remodeling along with sustained hyperpolarization of the associated vascular smooth muscle cells. A widespread loss of microvessels (rarefaction) and remodeling of larger vessels could contribute to elevated peripheral vascular resistance, abnormal tissue perfusion, and impaired organ function in chronic exposure to microgravity. A permanent reduction in vessel density mediated by structural degeneration of microvessels could have significant implications for countermeasures in microgravity environments and reloading strategies, since degeneration of microcirculatory vessels may lead to a sustained elevations in vascular resistance and alterations in tissue perfusion which would be refractory to therapy with vasodilator agents. However, the extent of microvascular rarefaction in different organs, the ability of microvascular rarefaction to be reversed, and the relative contribution of rarefaction to an elevated microvascular resistance and altered tissue perfusion remains to be determined. In the present project, we propose to specifically investigate both the mechanisms and the consequences of microvascular alterations in response to simulated microgravity and chronic gravitational loading. The central working hypothesis of the project is that rarefaction and vascular remodeling are continuous processes that occur in multiple vascular beds during exposure to microgravity and subsequent reloading, and that these changes lead to significant alterations in microcirculatory function. It is further hypothesized that the reduction in vessel density occurring during microgravity is mediated via one or a combination of factors, including elevated microvascular pressure or sympathetic nervous input. The specific aims of this project are: 1) To determine the extent of change in contractile and passive mechanical properties of isolated microcirculatory vessels following prolonged exposure to simulated microgravity; 2) To determine the functional consequences

of rarefaction and microvascular structural changes occurring during simulated microgravity, including effects on pressure and flow distribution in the microcirculation, arteriolar reactivity to vasoactive agonists, and tissue PO_2 distribution; 3) To determine the role of the sympathetic nerves, and altered perfusion pressure in contributing to vascular remodeling and rarefaction during simulated microgravity; and 4) To develop and analyze mathematical models and simulations of the dynamic process of microvascular alterations in simulated microgravity for prediction of experimental results and hypothesis testing. These aims will be investigated via acute and chronic experiments and histological studies in rats exposed to head-up tilt, head-down tilt, and tail suspension.

The main goals of this project are to evaluate the reduction in vessel density occurring during microgravity and to test the hypothesis that it is mediated via one or a combination of several factors including: 1) elevated microvascular pressure; 2) abnormal plasma angiotensin II levels; or 3), sympathetic nervous input. During this year Dr. Greene was invited to participate in the NASA life sciences advisory program in Dallas. At that meeting several of the findings from this work related to the influence of microgravity on the microcirculation were presented.

1. Shear Stress Suppresses Angiotensin Converting Enzyme Activity and Promoter Expression. Shear stress acting on endothelial cells *in vitro* has been shown to regulate the expression of numerous genes. We hypothesized that angiotensin converting enzyme (ACE), found predominately in the endothelium, would be suppressed by shear stress. Experiments were carried out both *in vitro* and *in vivo* to determine the effect of shear stress on the ACE promoter and converting enzyme activity. ACE promoter activity was measured in rabbit aortic endothelial cells stably transfected with 1300 bp of the ACE promoter coupled to the luciferase reporter gene (W3LUC). Converting enzyme activity was measured in cultured bovine pulmonary artery endothelial cells (BPAEC) which had been allowed to grow three days past confluence (0.31 ± 0.03 mUnits/mg). Shear stress was produced using a modified cone-plate apparatus, adapted for tissue culture dishes. Both sets of cells were exposed to shear stress levels from 0-20 dynes/cm^2 for 18 hours. Shear stress caused a suppression of converting enzyme activity (50 ± 8 %) and promoter expression (41 ± 9 %) compared to unsheared controls. The time course of converting enzyme activity and promoter expression was also studied following 2, 4, 8, 12 and 18 hours of shear. W3LUC cells exhibited a significant time-dependent inhibition of promoter activity over these periods. BPAEC converting enzyme activity was also suppressed during long term shearing (8, 12 and 18 hours) but exhibited a transient increase after 2 hours. In rat aorta, increased shear stress, induced by aortic constriction, reduced ACE activity by 40%. Taken together these studies demonstrate a potent link between hemodynamic stimuli and expression of endothelial ACE activity.

2. Tissue PO_2 and Arteriolar Dilation During Parent Vessel Occlusion in Cremaster Muscle of Spontaneously Hypertensive Rats (SHR). The goal of this study was to determine whether increases in tissue PO_2 affect the dilation of third order cremasteric arterioles of SHR and normotensive Wistar Kyoto (WKY) rats during the increase in flow which occurs during downstream occlusion of the second order parent arteriole (PA). During superfusion of the tissue with physiological salt solution equilibrated with 0% O_2, arteriolar dilation during PA occlusion was significantly larger in WKY than in SHR, even though tissue PO_2 (measured with O_2 microelectrodes) was significantly lower in SHR. During 5% O_2 superfusion, tissue PO_2 increased and was not significantly different in SHR and WKY. Arteriolar dilation during PA occlusion was unaffected by elevation of superfusate PO_2, and was still significantly larger in WKY. These observations suggest that elevation of tissue PO_2 does not override arteriolar dilation during PA occlusion and that dilation of SHR arterioles during PA occlusion is impaired not only at the lower resting tissue PO_2 in the hypertensive animals, but also during conditions of increased O_2 availability.

Significance: Some of the most striking changes that develop in certain organs during exposure to microgravity and subsequent reloading occur in the microcirculation. Studies from a number of groups have demonstrated atrophy of the microvasculature and changes in the functional properties of blood vessels which appear to impact directly on organ function. Recent studies by our group indicate that

chronic head up-tilt results in vascular remodeling along with sustained hyperpolarization of the associated vascular smooth muscle cells. A widespread loss of microvessels (rarefaction) and remodeling of larger vessels could contribute to elevated peripheral vascular resistance, abnormal tissue perfusion, and impaired organ function in chronic exposure to microgravity. Furthermore, a permanent reduction in vessel density mediated by structural degeneration of microvessels could have significant implications for countermeasures in microgravity environments and reloading strategies, since degeneration of microcirculatory vessels may lead to a sustained elevations in vascular resistance and alterations in tissue perfusion which would be refractory to therapy with vasodilator agents. However, the extent of microvascular rarefaction in different organs, the ability of microvascular rarefaction to be reversed, and the relative contribution of rarefaction to an elevated microvascular resistance and altered tissue perfusion remains to be determined. In the present project, we have demonstrated that simulated microgravity results in a loss of microvessels in skeletal muscle of a magnitude similar to that which occurs in hypertension. It remains to be seen if these changes lead to significant alterations in microcirculatory function.

Studies are planned to determine the extent of microvascular rarefaction and the changes in contractile and passive mechanical properties of resistance vessels and *in situ* microvessels during prolonged exposure to simulated microgravity. In order to do this, we will examine the extent and time course of microvascular rarefaction, the sensitivity of microvessels to vasoconstrictor and vasodilator stimuli, and the changes in passive mechanical properties of microvessels from rats exposed to simulated microgravity.

The central theme and fundamental hypothesis of our Program is that arterial pressure is importantly controlled by the renal-body fluid system which determines sodium and water balance and that abnormalities in fluid balance influences the systemic vascular tone through the physical factors of pressure and wall shear force. Based on studies carried out in the Program by Drs. Lombard and Greene, our attention has focused on the structural changes seen in skeletal muscle and other tissues in volume-expanded and other models of hypertension in which there is a reduction in the density of microvessels (microvascular rarefaction). Mathematical network models developed by our group have indicated that the degree of the rarefaction observed in some skeletal muscles in hypertension can contribute to increases in microvascular resistance, increases in the heterogeneity of blood flow, reduced oxygen delivery, and impaired muscle performance. Neither the functional importance of microvascular rarefaction nor the mechanisms which trigger these responses are completely understood.

This research aims to determine the extent of rarefaction in different regions of the body and the time course and reversibility of this process. It aims also to determine the role of the renin-angiotensin system, the sympathetic nerves and elevated perfusion pressure in contributing to rarefaction during hypertension and changes of salt intake, both of which appear to independently influence the density of microvessels. The functional consequences of these microvascular structural changes on pressure and flow distribution in the microcirculation and the arteriolar reactivity to vasoactive agents and tissue PO_2 distribution will also be determined.

Although not a direct goal of this research, the development of therapeutics or protocols for reducing the microvascular changes that occur in hypertension is a long term goal. Understanding the fundamental mechanisms that contribute to microvessel loss and altered function in simulated weightlessness will help us to understand the role of orthostatic loading on Earth.

Since small resistance arteries and arterioles are the major controllers of vascular resistance and tissue blood flow, understanding their function is of basic biological importance. The studies of isolated small arteries and the *in situ* microcirculation of animals subjected to simulated microgravity in the present project will provide direct information regarding alterations occurring in the smallest blood vessels of skeletal muscle during acute and prolonged exposure to reduced gravitational load. Degenerative structural alterations of the microcirculation could also contribute to the cardiovascular complications following exposure to microgravity. Structural degeneration in the microcirculation could include both

a loss of microvessels (microvascular rarefaction) and structural degeneration of endothelial and vascular smooth muscle cells in the remaining microvessels. These degenerative structural changes could adversely affect the ability of the microcirculation to actively control pre- and postcapillary resistance, venular capacitance, and tissue blood flow. Reductions in vessel density could also compromise the ability of the microcirculation to deliver O_2 and nutrients and remove waste products from the tissue by decreasing the number of exchange vessels in the tissue and increasing the diffusion distance for O_2, nutrients and waste products as a result of a greater intercapillary distance.

In this research we will study the stress of simulated microgravity and gravitational loading. It has long been known that cardiac and vascular deconditioning occurs during prolonged exposure to microgravity. Considerable attention has been focused on these events in larger vessels and in the reflex control of the circulation. Over the last several years, our attention has focused on structural changes seen in skeletal muscle and other tissues during hypertension and other abnormal situations. One of these changes is a process called microvascular rarefaction, which is a degradation and loss of blood vessels in the microcirculation. Mathematical models developed by our group have indicated that the degree of rarefaction experimentally observed in skeletal muscle under some circumstances can contribute to increases in microvascular resistance, increases in the heterogeneity of blood flow, reduced oxygen delivery, and an overall impairment of organ function. Our experimental studies have demonstrated that situations which cause changes in body fluid volumes such as hypertension (reduced renal mass, RRM) and high Na intake cause microvascular rarefaction. Studies from our laboratory have shown that chronic head-up tilt results in vascular remodeling along with sustained hyperpolarization of the associated vascular smooth muscle cells. Preliminary studies have also shown that 3 days of head-down tilt is associated with rarefaction of microvessels in the cremaster muscle which is of a similar magnitude to that seen in hypertension. However, neither the functional importance of microvascular rarefaction nor the mechanisms which trigger this response are completely understood. Based on our experimental and theoretical studies to date, we have developed 3 general hypotheses: 1) That rarefaction and vascular remodeling is a rapidly occurring and progressive process of structural alteration which occurs in the microcirculation of multiple vascular beds in response to microgravity; 2) That elevated perfusion pressure and enhanced sympathetic neural input stimulated by volume shifts can contribute to microvascular rarefaction and remodeling during chronic gravitational load; and 3) That structural alterations occurring in the microcirculation affect the hemodynamic and functional properties of the microcirculation, including microvascular flow distribution, arteriolar reactivity to vasoactive agonists, and tissue PO_2 distribution.

Orthostatic intolerance and reduced exercise capacity are well known complications following space flight or prolonged bed rest. Both of these conditions may be related to alterations in the structural and functional properties of the peripheral vasculature. These changes in the structure and function of the microcirculation also occur during the development of hypertension, during normal aging, and during periods of high salt ingestion. Understanding how diet and behavior impact on microcirculatory function will allow us to develop techniques to reduce the impact of these changes on organ function.

Publications, Presentations, and Other Accomplishments:

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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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Task Description:

In the original September 1992 proposal, a two-part experimental protocol was designed. Part A was designed to test that a single cycle of properly timed exposure to bright light and darkness is able to induce sufficient physiologic and psychologic adaptation of the circadian sleep-wake cycle to allow astronauts to function effectively on the day of launch (hypothesis 1). Six male and six female subjects were to be exposed to a single cycle of bright light, ordinary indoor light, and darkness. Part B was designed to test the stability of this response in an environment impoverished of circadian time cues (hypotheses 2) and the gradual regrowth of circadian amplitude following the bright light stimulus (hypothesis 3). After an 8-h recovery sleep from CR2, the 12 subjects would undergo a third CR to monitor extended responses to the resetting stimulus. However, since the grant proposal was submitted in 1992, data we have gathered indicate that it is critical that there be a control group in order to accurately quantify the stability of the circadian phase and amplitude following exposure to the resetting stimulus. Therefore, as previously reported, we have replaced the gender comparison group with a two-part control protocol which was identical to the experimental protocol described above, except for the timing of the bright light exposure. Computer simulation using Kronauer's mathematical model of the resetting effect of light on the human endogenous circadian pacemaker reveals that an 8-h light stimulus administered 2.5 h after the minimum of the fitted core body temperature curve should yield a phase advance of approximately 2.7 hrs and a 38% reduction in circadian amplitude, which we therefore plan to use in this protocol. We plan to study six subjects in each protocol for a total of twelve subjects. We also plan to recruit several female subjects in order to gather preliminary data on gender differences. Overall, our aims at this component of the project remain unchanged from the original proposal. In addition, in 1993, we proposed that we carry out studies of the effect of intermittent light vs continuous light exposure on resetting the human circadian pacemaker. These studies, which were begun during a previously supported NASA grant, were completed during year 1 of the current grant period.

Data derived from continuation of the previously supported NASA grant which was completed in Year 1 of the current grant period have revealed that bright light can substantially phase shift the human circadian pacemaker even when the light exposure is interrupted by recurrent 19- to 43-minute intervals of complete darkness. Thirty-two healthy young males aged 18-30 years were exposed to three consecutive daily, 5-hr stimuli which consisted of one of the following duty schedule patterns of bright-light vs complete darkness: (1) continuous bright light exposure (~9,500 lux); (2) intermittent bright light exposure interspersed with complete darkness (three uniformly spaced darkness intervals of 43 minutes each, for which the light represented 57% of the 90 minute repeat cycle); (3) intermittent bright light exposure interspersed with complete darkness (12 uniformly spaced darkness intervals of 19 minutes each, for which the light represented 23% of the 25 minute repeat cycle; and (4) continuous darkness (~0.03 lux). The mean (±SEM) phase advance shifts observed were 4.69 ± 0.42 hours, 3.54 ± 0.52 hours, and 2.17 ± 0.59 hours after exposure to the continuous bright light, the intermittent 57% bright-light duty schedule, and the intermittent 23% bright-light duty schedule, respectively. A phase delay of -1.13 ± 0.43 hours was observed in the group exposed to continuous darkness, consistent with the slightly longer-than-24 hour period of the endogenous circadian pacemaker. ANOVA for multiple between group comparisons revealed that: 1) the phase advance shifts in all light-exposed groups were significantly different from the phase delay observed for the group of subjects exposed to darkness; 2) the phase advances observed for the 57% bright-light/darkness group were not significantly different from those observed in the group exposed to continuous bright light; and 3) the phase-advances observed for the 23% bright-light/darkness group were statistically different from those of the other light-exposed groups ($F_{3,27}=27.51$, $p=.0001$).

Therefore, even when the bright light exposure occurred in only 57% of the repeat cycle, 80.5% of the resetting response was preserved when compared with the 5-hr uninterrupted stimulus. Likewise, when the bright light exposure occupied only 23% of the repeat cycle, 53.8% of the resetting response was preserved when compared with the 5 hour uninterrupted stimulus. Kronauer and Czeisler have proposed two alternative (and functionally equivalent) mathematical models for a dynamic signal conditioner which intervenes between the light and the circadian pacemaker.

In the more fundamental model, darkness is a state during which potential effector elements are readied for phase shifting, to be activated on the next light exposure. In the second model, phase shifting continues with declining strength during darkness. Both models embody a saturation response as light intensity is increased. With proper timing of "on" and "off" it may be possible to design low duty cycle patterns which are almost as effective as those with continuous exposure.

Previous studies of astronauts have documented the presence of circadian rhythm 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, together with erratic exposure to light among astronauts, is thought to be primarily involved in the physiopathology of 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 prelaunch 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 carried out during the first year of support have major implications for understanding the effect of intermittent and/or erratic exposure to light among astronauts during space flight. Better understanding of the basic mechanisms underlying this responsiveness to intermittent light is necessary to ensure stable entrainment of the circadian system during space flight. The results

of this first year of support will also lead to the refinement of the Kronauer 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. During years two and three of the present grant, we plan to test the stability of the resetting effect of a single cycle of light exposure in an environment impoverished in light exposure. We predict that a single cycle of exposure to bright light and darkness 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 and the common man.

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Dynamics of Neural Adaptation to Altered Gravitational Conditions Revealed by Neurobehavioral, Neurochemical, and Morphological Studies

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FY 1995 Funding: $163,000

Students Funded Under Research: 4

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 1G 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 2G? 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 2G 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.

Experiments were conducted to begin to answer the first two of the four research questions listed in the task description, while we also started the refinement of procedures for addressing the third and fourth questions in the list. In addition, several experiments were conducted to assess the effects of increasing either G level or duration of exposure in an attempt to magnify morphological and neurochemical modifications elicited by hyper-G exposure. If these changes can be magnified, our ability to detect them will thus be enhanced and therefore our assessment of neural mechanisms involved in adaptation to altered G will have greater validity. In addition, having baseline behavioral data on different G levels and exposure durations will allow us to assess efficacy of drugs as facilitators of adaptation under several different conditions that differ in the degree of adaptation required. To enhance our understanding of the vestibular and neuromuscular contributions to sensory-motor adaptation to altered G, we have initiated studies involving exposures to ototoxic drugs (affecting primarily the vestibular system) and to hindlimb suspension (HLS - affecting primarily the neuromuscular system). Thus we can evaluate behavioral adaptation when the initiating condition affects primarily the vestibular system (ototoxic drugs), the neuromuscular system (HLS), or a combination of the two (altered G). This information will be useful in evaluating the site of action of drugs which facilitate the adaptation process and in selecting additional drugs for future testing, as well as for understanding the process of neural adaptation to altered G.

Although a great deal of behavioral data and tissue are still awaiting processing and analysis, findings from studies conducted during FY95 include the following: 1) There appears to be no difference in the degree of disruption of vestibular function (as measured by behavioral assessment of righting reflex and orientation) elicited by 2G vs 3G, either in terms of initial magnitude of disruption or in terms of time taken to recover normal vestibular function following centrifugation. 2) Animals exposed to 3G showed a significant diminution of GABA-ergic immuno-reactivity in the somatosensory cortex and in certain areas of the cerebellum, but no changes in lumbar spinal cord or in vestibular nuclei. (Note that GABA was assessed in this experiment rather than TRH or Substance P, as originally proposed. This neurotransmitter is often co-localized with the latter neuropeptides and is easier for us to assess, thus more appropriate in this initial exploratory experiment.) 3) In a study in which animals were repeatedly exposed for 7-day periods to 2G, readaptation of a "restricted" group and an "activity" group of animals was assessed. Preliminary evaluation of data suggests that the greater the degree of sensory-motor activation during readaptation, the more rapid the readaptation process. Similarly, preliminary data indicate that the more experience the animals have with the 2G environment, the more rapidly they readapt following the chronic 2G exposures. However, additional experimentation and the full data analysis must be completed to confirm these observations. 4) In a study comparing the effects of 14 days of hindlimb suspension with those of 14 days of 2G exposure on air-righting and orientation during swimming, we found that air-righting recovers more rapidly (within 2 days) following suspension than following 2G exposure. On the other hand, orientation shows no disruption following suspension, while it is disrupted for the first 24 hours following 2G exposure. These results suggest that air-righting has a neuromuscular component in addition to the vestibular component, and that orientation may be a more purely vestibular response.

Work on the otolith-spinal reflex test to monitor gain in the otolith portion of the vestibular system is progressing well, and this experiment should be conducted ahead of schedule. Studies to assess the efficacy of various drugs as facilitators of the readaptation process will be initiated during this fiscal year, and although this is later than originally scheduled, we anticipate completing the studies on schedule, given continued availability of the centrifuge.

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.

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Postural Effects on PTH, Calcium, and Skeletal Dynamics

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Expiration: 4/97

FY 1995 Funding: $111,704

Students Funded Under Research: 1

Task Description:

Marked bone demineralization and severe hypercalciuria resulting in an increased risk of stone precipitation are two salient features of the weightless state. The mechanisms responsible for these changes are unclear. There is an increasing body of evidence supporting an anabolic effect of PTH circadian rhythms on bone remodeling. In addition the essential role of the kidney in calcium balance is undisputed. Previous studies unraveled a number of abnormalities in response to the weightless state, including disturbances in the amplitude and period of various circadian rhythms and alterations in renal hemodynamics and sodium homeostasis. The overall goal of this proposal is to evaluate the effect of the semirecumbant posture, as one dimension of the weightless state, on calcium and skeletal dynamics.

Specifically we will test the hypothesis that the semirecumbant posture 1) induces a blunting in the amplitude of PTH circadian rhythms and alterations in its phase, and 2) disturbs renal calcium handling, thus resulting in a negative bone remodeling profile. These studies will bring a new dimension to our understanding of some of the mechanisms responsible for both space flight induced and idiopathic osteoporosis and nephrolithiasis, thus allowing the development of novel countermeasure programs to prevent these processes.

In the first Specific Aim we will test the hypothesis that the semirecumbant posture induces a blunting in the amplitude of PtH circadian rhythm and alterations in its phase resulting in a negative bone remodeling profile. Since the start of the grant I have held several work meetings with Dr. Beth Klerman, the fellow who will screen and schedule subjects for studies, and the subject recruiter to discuss the eligibility criteria, the consent form, the protocol design and implementation. In addition, I have met with the nurse in charge and the technicians of the Intensive Physiologic Monitoring Unit, the Research unit where the circadian studies will take place, to discuss the protocol implementation in detail. This includes the protocol orders, phlebotomy techniques and schedule, urine collection schedule and sleep and body temperature monitoring. We have also covered in detail the time table for sample handling and destination. Starting Dec 95, we have screened 30 subjects on the telephone, 20 were eligible for our study and 10 are scheduled for an evaluation including a physical examination and routine blood work. We do not anticipate any changes in our experimental design and expect to complete 10 studies by the end of July as originally planned.

In the Second Specific Aim we are to evaluate the effect of posture per se on renal calcium handling. According to the grant time table we will study 16 subjects for the renal studies during the second year. However, during our first year we have completed considerable ground work to refine the renal calcium clearance protocol. Due to the important effect of PTH on renal calcium handling, we modified the original protocol to ensure stable PTH levels during the calcium clearance protocol, We have studied 6 subjects in our inpatient Clinical Research Center. They received a constant PTH infusion (Parathar PTH 1-34) at a dose of 0.2U/kg 12 hours prior to and continuing throughout the renal clearance experiment. This infusion results in intact PTH levels below 10pg/ml by 12 hours and N-terminal PTH levels in the upper limit of normal. For the first time, we we were able to demonstrate a tight sigmoidal relationship between serum ionized calcium and urinary Ca/Cr ratio with a set- point of 1.5 mMol/L. There was no change in either GFR or renal blood flow (assessed using PAH and inulin clearances) in response to changes in serum ionized calcium levels. This protocol was performed in the semirecumbant posture. During the coming academic year we will implement this protocol in the upright and the semirecumbant posture on two consecutive days in 16 subjects as originally planned.

This protocol will shed important light on the mechanism of immobilization hypercalcemia, hypercalciuria and bone loss. If we demonstrate that the semirecumbant posture results in a blunting of PTH amplitude and results in a catabolic bone remodeling profile, this would lead to the design of preventive and/or treatment strategies aimed at enhancing PTH rhythm and amplitude. Similarly, if we demonstrate renal calcium wasting in the semirecumbant posture and are able to further delineate the specific site for such effect, we could use medications targeted at the specific site to prevent calcium excretion and therefore a negative calcium balance state. These would be therapies applicable to immobilization hypercalcemia. 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 annual expenditure of 10 billion dollars annually, a number that is on the rise due to the increasing age of the elderly population. Our protocols will not only shed important light on the mechanism of immobilization induced bone loss, but also on osteoporosis in general and thus will allow the development of innovative prevention/treatment strategies for this common debilitating and costly disease.

Cardiopulmonary Hemodynamics in Microgravity

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Project Identification: 199-14-17-06
Solicitation:
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Students Funded Under Research: 13

Task Description:

Ground based studies in our and other laboratories 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 hypothesizes were that removing gravity would result in: 1) an increase in end-diastolic volume, however 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). 2) The increase in pulmonary blood flow and volume would be accommodated by a decrease in pulmonary resistance caused by vasodilitation 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.

During the preceding year, we have concentrated our studies on the measurement of cardiac and pulmonary blood flow and volume. We have conducted two series of experiments using head down tilt in combination with lower body pressure and graded head out water immersion as simulations of earth's

gravity and microgravity. Preceding these experiments, we have developed the instrumentation to apply these stresses in a clinical nuclear medicine facility and developed the hardware and software to make the necessary measurements. Our studies consisted of 8-10 subjects who were given physicals and completed the studies. Measurements of pulmonary blood flow were made using the Farhi CO_2 re-breathing technique. Measurements of cardiac blood volumes and compliance were made from nuclear imaging using gaited-pooled samples over a five minute period. Pulmonary blood volume was determined from nuclear imaging the right lung from the posterior view over a one minute period. From these experiments we have demonstrated that: 1) end-diastolic volume and end-systolic volume increase as the simulated effects of gravity were decreased. The stroke volume increased as the increase in end-systolic volume was less than the increase in end-diastolic volume. Cardiac output increased as the increase in stroke volume was greater than the decrease in heart rate. The time to end-diastolic volume and the rate of change of volume were inversely related to the simulated gravity. These data suggest that there is a progressive decrease in sympathetic tone and increased cardiac compliance as simulated gravity is progressively reduced. These data appear to be directly related to observations made in space. 2) Pulmonary blood flow and volume progressively increased as simulated gravity was progressively decreased. The blood volume was distributed such that the base of the lung had the greatest volume and the volume decreased in segments toward the top of the lung (a heterogeneous distribution). Increasing the lung volume did not effect the distribution of the volume, only the amount of blood in each segment, thus the lung appeared to still have a heterogeneous distribution. Although this suggests that the pulmonary blood vessels were engorged and that the distribution of lung blood flow and volume are not gravity dependent, further studies are needed to confirm these observations. It is clear that there was a reduction in pulmonary resistance with a decrease in simulated gravity, which could influence the relationship between cardiac output and central venous pressure.

Our upcoming experiments will focus on determination of capillary recruitment and total lung capillary volume (engorgement) in the two simulated gravity conditions described above. In addition, we are developing the technology to determine the lung volume distribution under conditions of increased gravity in the human centrifuge.

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 our experiments add support to space flight data. These 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.

Publications, Presentations, and Other Accomplishments:

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Limb Muscle Function with Unloading and Countermeasures

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FY 1995 Funding: $132,562

Students Funded Under Research: 6

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 unloaded (HU) rat model to study the cellular properties of individual fibers isolated from the soleus muscle. In this funding period, we completed studies designed to determine the effects of hindlimb unloading (HU) on fiber force, stiffness, and Ca^{2+} sensitivity, and began work evaluating various exercise countermeasures, and the mechanisms responsible for the HU-induced increase in maximal shortening velocity (V_o) and reduced fiber tension. During the first three weeks of HU, fiber diameter and peak force (mN) showed a progressive decline, while the maximal shortening velocity (V_o) increased. Our results showed that the decline in force was the result of the combined effects of fiber atrophy and a disproportionate loss of contractile proteins. In addition to force, peak tension (kN/m^2) also declined with HU. We hypothesize that this was caused by the reduced contractile protein which in turn increased the spacing between the myofilaments. The increased lattice spacing might explain not only the loss of tension, but also the increased fiber V_o. We are currently conducting experiments to test this hypothesis.

We have evaluated 2 countermeasures (standing and ladder climbing), and developed the technology for studying weight lifting. The intermittent standing reduced the loss of relative soleus mass by 22% and attenuated the alterations in the type I fiber diameter by 36%, peak force by 29%, and V_o by 48%. The treatment had little or no effect on tension or power. The ladder climbing countermeasure was more effective than the standing in preventing the deleterious effects of HU. For example, peak tension averaged 139 ± 3, 116 ± 5, and 133 ± 4 $kN \cdot m^{-2}$ for the control, HU and HU plus ladder climbing groups, respectively. The effectiveness of the ladder climbing is perhaps best demonstrated by

evaluating peak power. In this study HU reduced peak power from the control value of 15.7 ± 0.8 to 9.3 ± 0.6 $\mu N \cdot FL \cdot s^{-1}$, while the HU plus ladder climbing group showed a peak power of 13.9 ± 0.7 $\mu N \cdot FL \cdot s^{-1}$. It is clear from our results that heavy resistive exercise bouts conducted in short and frequent sessions (10 min, 4/day) is an effective countermeasure. Our results suggest that an optimally designed high resistive exercise program should (without supplemental hormone treatments) be able to prevent the deleterious functional changes associated with zero gravity space travel. A major question remaining is whether such a program can also prevent all cell atrophy, and thus totally protect against muscle weakness and the reduced work capacity associated with zero g space travel.

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 the atrophy process 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.

Publications, Presentations, and Other Accomplishments:

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Effect of Microgravity on Vascular Cell Function

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Funding:

Project Identification: 199-08-17-72/P01HL2958212 Solicitation:

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Joint 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 Wand 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: (I) 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. (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 (FGE;)-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.

Our simulation of microgravity involves temporal neutralization of the gravity vector by low-speed rotation of sealed tissue culture dishes containing adherent endothelial cells or monocytic cells. The experiments were facilitated by the customized construction of a low-vibration, variable speed rotation platform by ATR Research Equipment (Laurel, MD). This platform permits rotation of samples from 8 to 50 rpm. Several technical obstacles were presented by these long-term studies, especially those concerning cell viability. In particular, air bubbles released from the medium caused cell damage as assessed visually or by incorporation of [3H]leucine into protein. Dr. Liming Wang, Research Associate on this Project, found that Parafilm seals irreversibly trapped small air bubbles. The use of a Parafilm seal thus completely prevented the interaction of air bubbles with cell surfaces and permitted us to perform long-term studies on cells without loss of viability.

We first examined the effect of simulated microgravity on basal (i.e., unstimulated) EC movement. Bovine aortic EC were grown to confluence in plastic tissue culture dishes and a razor wound was made to initiate cell movement. Triplicate wells were rotated at 8 rpm at 37°C for 24 h and then the cultures fixed and stained for quantitation of wound-induced cell movement by computer-assisted image analysis. As controls, parallel cultures were similarly wounded and incubated under the following conditions: (1) "mixed" controls were shaken upright on an orbital mixer (to show that effects observed in the "microgravity" wells were not simply due to mixing), (2) "mixed, inverted" controls were shaken upside-down on an orbital mixer (to determine if "microgravity effects" were due to the time that the cells spent upside down, rather than to neutralization of the gravity vector), and (3) "stationary" controls for historical comparison to previous data by us and others. The initial experiments revealed that the cultures subjected to simulated microgravity and all control cultures exhibited nearly identical rates of movement. In addition, the rates of total protein synthesis, measured by incorporation of [3H]leucine into protein, were identical for all treatments. These early experiments were disappointing for obvious reasons, but did clearly demonstrate the usefulness of our system and the feasibility of our approach.

In subsequent experiments we tested additional parameters, namely the effects of agonists and platform rotation speed, on EC migration under conditions of simulated microgravity. We observed that at higher speeds (20 rpm), EC migration was stimulated by serum to a much less extent than under all control conditions. In one experiment, there was essentially no stimulation by serum under "microgravity" conditions while serum stimulated migration 2-fold under the control conditions. In two other experiments the effect was somewhat less dramatic - the serum-stimulation under "microgravity" conditions was about half that under the control conditions. Similar results were not observed at low-speed (8 rpm) platform rotation; serum-stimulated migration was essentially identical under "simulated microgravity" and control conditions. Interestingly, the differential results seen for serum-stimulated migration were not seen for basic fibroblast growth factor (FGF)-stimulated migration. We do not understand this different response of serum- and basic FGF-stimulated cells, but have preliminary data showing that these factors stimulate migration by different signal transduction pathways, and that the morphology of the stimulated cells is very different. We are currently examining these results in more detail by time course experiments and studies involving a large range of rotational velocities.

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.

Effects of Artificial Gravity: Central Nervous System Neurochemical Studies

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Funding:

Project Identification: 199-16-17-14
Solicitation:

Initial Funding Date: 5/95
Expiration: 5/96

FY 1995 Funding: $100,000
Students Funded Under Research: 8

Task Description:

The purpose of the present proposal is to assess chemical and morphological modifications occurring in muscle receptors and the central nervous system of animals subjected to hypergravity (2 X Earth gravity, or 2G) produced by centrifugation. The underlying hypothesis of the project is that disturbance (i.e., alteration) of normal gravity should alter afferent (sensory) information sent to the central nervous system by muscle receptors. Those changes, in turn, will affect the chemical behavior of neurons and glial cells of the projection areas of the cerebral cortex that are related to inputs from those muscle receptors (e.g., cells in the limb projection areas).

Animals will be subjected to 2G for 4 or 14 days after which they will be euthanized or anesthetized and then fixed by perfusion. Neural cell adhesion molecule (N-CAM) immunocytochemistry and electron microscopic techniques will be employed for the study of muscle spindles. Immunocytochemical procedures for the study of neuroactive substances (e.g., g-aminobutyric acid, or GABA, and neuropeptides), and neurotransmitter receptor binding techniques for localization of receptors (e.g., GABAA) with the light microscope will be applied. The principal structures to be studied include lumbar spinal cord, the somatosensory cortex (in particular the hindlimb representation area) and the motor cortex.

We have shown previously that significant changes occur in air-righting, orientation, and locomotion of rats chronically exposed to hypergravity produced by centrifugation. To investigate neural mechanisms underlying these changes, rats exposed were exposed to 3G for 14 days and g-aminobutyric acid (GABA) immunoreactivity was studied in areas of the brain and spinal cord that are involved in motor control. GABA-ergic immunoreactivity was studied in cerebellum (anterior vermis, flocculus), lateral vestibular nucleus, somatosensory cortex (hindlimb representation), and spinal cord (dorsal horn).

Immunoreactivity of GABA-ergic cells was reduced in hindlimb representation of the somatosensory cortex and in the cerebellum. In somatosensory cortex there was a marked decrease in reactivity of GABA-ergic cells and their terminals in animals exposed to 2G. In the cerebellum, exposure to 2G led

to decreased immunoreactivity in local circuit GABA-ergic cells ("basket" cells) and their terminals that surround Purkinje cell bodies. In addition, hypertrophy of neuroglial cells (astrocytes) of the cerebellar gray (von Bergmann cells) and of the white matter occurred in rats exposed to 2G. No differences due to exposure to 3G were identified in GABA immunoreactivity in GABA-ergic cells or in terminals in the dorsal horn of spinal cord. Neither were changes identified in small GABA-ergic cells of the lateral vestibular nucleus or in the density of GABA-ergic terminals surrounding Deiter's neurons.

Since the decrease in GABA immunoreactivity was seen in some, but not all areas investigated, it appears that this effect is confined (at least after 14 days of centrifugation) to certain functional systems, and it is not a generalized effect of chronic exposure to hypergravity on the central nervous system. Exposure to hypergravity appears to affect local circuit GABA-ergic neurons that modulate the discharge of output neurons (pyramidal and Purkinje cells) of command motor centers (cerebral and cerebellar cortex). These results suggest our previous demonstration of changes in locomotion and righting reflex after exposure to hyper-G could be brought about by altered proprioceptive feedback from muscle receptors. The decrease in GABA immunoreactivity may be reflecting changes in modulatory functions of the transmitters influencing neurons that generate central command signals to the neuromuscular system.

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 (2G). 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.

Publications, Presentations, and Other Accomplishments:

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D'Amelio, R., R. Fox, L-C. Wu, N. Daunton "Quantitative changes of GABA-immunoreactivity in the hindlimb representation of the rat somatosensory cortex after 14-day handlimb unloading by tail suspension." Meeting of the Society for Neuroscience, San Diego, CA, Nov. 1995.

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Sergutina, A., L. Gershtein, F. D'Amelio, N. Daunton, I. Krasnov "Some cytochemical features of the motor system of the rat brain after space flight." Byulleten Eksperimental'noi Biologii i Meditsini (Bulletin of Experimental Biology and Medicine), Russia, 119 (3), 288-290 (1995).

Sergutina, A., L. Gershtein, F. D'Amelio, N. Daunton, I. Krasnov "Cytochemical analysis of the somatosensory cortex and caudate nucleus of the rat brain after 9-day space flight." X Conference Space Biology and Aviaspace Medicine. Moscow, Russia, 158, 1994.

Circadian Rhythms in Rhesus: Gravity, Light & Gender

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-18-17-18

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $223,530

Students Funded Under Research: 5

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.

This project examines the effects of exposure to altered lighting and gravitational environments on the circadian rhythms of body temperature, activity, drinking and various performance parameters of male and female rhesus monkeys. Temperature and activity are monitored by telemetry and drinking by an electronic contact circuit. The psychomotor test system (PTS), developed at the University of Georgia Language Research Center, is a computer-based video task system that enables us to test short term memory, hand-eye coordination, and other performance parameters. Successful completion of a video task is rewarded with a pelletized diet. The PTS is designed to provide all daily food to a fully trained subject. During FY95 we developed and refined individual psychomotor test stations to be used for 1G and 2G studies. The PTS stations for the 1G studies are stand-alone systems that are contained on individual carts that are attached to the front of the animal's home cage. The carts can be easily removed for daily animal husbandry tasks. The video monitor is placed at eye level for the animal and the joystick is centered below the screen. The feeder delivers a pellet into a food cup easily accessed by the animal.

The animals are individually housed in standard primate cages in a environment controlled room at the California Regional Primate Research Center (CRPRC). In order to maintain social interaction, the cages are set up to provide visual and auditory contact between the animals. Entry into the room is strictly controlled in accordance to the project's protocol. The animals are checked daily for health and

reported to the CRPRC veterinary staff. Additionally, food intake is closely monitored and the animals are weighed every two weeks.

In order to perform the 2G studies, the rhesus will be maintained on a 6 meter diameter centrifuge. We have developed a module that will allow us to record PTS, video, temperature, activity and drinking continuously, as well as to collect urine, from rhesus under these conditions. A standard vivarium cage fits within the module and houses the animal. The orientation of the PTS is the same as it is in the 1G station.

We have trained 8 male rhesus in all sixteen PTS tasks. These animals are fully trained (i.e. able to perform all tasks to success criteria); all their caloric needs are met through the operation of the PTS system. We are now able to record their performance on various tasks continuously and are beginning a constant light study. In addition, we have begun training 4 female rhesus in the use of the PTS. The females are housed in a separate room at the CRPRC, specifically for PTS training. Four additional female animals will begin to train as the study progresses for a total of 8 males and 8 females. All the animal subjects are age-matched and will be starting the project at approximately the same age. They were also screened for any health problems before being accepted to the study. We have found that the time required for training varies between individual animals and ranges from 5-10 months. We have collected data from all animals during their training phase. This data will be analyzed to determine if there are any gender based differences in the manner in which animals acquire proficiency with the PTS system.

We have begun to develop programs designed to allow us to analyze the behavioral data using in house circadian analysis programs. We are writing a computer program that will analyze the PTS data. Number of trials and average response time will be computed in 10 minute increments for psychomotor tasks. Short term memory tasks will be analyzed for number of trials attempted and number of correct tasks every 10 minutes. The output of this program will be compatible with the lab's rhythm analysis program, so that rhythms of psychomotor and memory based task performance can be analyzed.

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 women astronauts.

Publications, Presentations, and Other Accomplishments:

Fuller, C.A., T.M. Hoban-Higgins and D.M.Murakami "Effects of altered gravitational environments on primate circadian rhythms." Biol. Rhythm Res., 26(4), 391 (1995).

Intercompartmental Fluid Shifts in Response to Postural and Gravitational Forces

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-14-17-03

Solicitation:

Initial Funding Date: 10/93

Expiration: 10/96

FY 1995 Funding: $80,000

Students Funded Under Research: 0

Task Description:

No additional data was provided by the investigator for this research.

Role of Integrins in Mechanical Loading of Osteoblasts

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Funding:

Project Identification: 199-26-17-15 Solicitation: 93-OLMSA-07

Initial Funding Date: 7/95 Expiration: 6/98

FY 1995 Funding: $161,771 Students Funded Under Research: 0

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 (Morris, 1990), 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 will conduct both *in vitro* and *in vivo* studies using the rat as a model to test these ideas. Initial studies will reveal whether mechanical loading regulates expression of specific integrin and ECM components associated with discrete stages of osteoblast differentiation. Later studies will test the hypothesis that integrins transduce signals generated by mechanical force that ultimately alter osteoblast function. We propose the following specific aims: Specific Aim 1: Determine if in vivo changes in weightbearing induced by exposure of the growing rat to hindlimb unloading or

microgravity regulate the type, amount, or adhesive activity of integrins expressed by cells of the osteoblast lineage. Specific Aim 2: Determine how changes in mechanical loading affect integrin expression during progressive osteoblast differentiation *in vitro*, using both primary rat osteoblasts and multipotential C26 cells exposed to stretch or microgravity. Specific Aim 3: Test the hypothesis that specific integrin-ECM interactions mediate mechanical stretch-induced changes in osteoblast function *in vitro*.

During the first six months work on this grant, we developed a suitable cell culture model for investigating the role of integrins in the response of osteoblasts to mechanical stimulation and we have shown that a component of the extracellular matrix, fibronectin, plays a functionally significant role in osteoblast differentiation. We selected primary fetal rat osteoblasts as a culture system for study, and evaluated the time course of differentiation using Northern analysis of mRNA expression for several genes characteristic of maturing osteoblasts (type I collagen, alkaline phosphatase, osteopontin and osteocalcin) and histochemical staining for extracellular matrix protein expression and mineralization. Based on the finding that the sequence of morphological and biochemical changes occurring during differentiation of primary osteoblasts resembles the process of bone formation *in vivo*, we conclude that these cells will provide an appropriate system for the study of mechanical stimuli. In addition, we have shown that fibronectin, an extracellular matrix protein produced by osteoblasts, regulates both morphological and biochemical features of osteogenic differentiation.

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.

Publications, Presentations, and Other Accomplishments:

Globus, R.K., Moursi, A., Lull, J., Zimmerman, D., Doty, S.B., and Damsky, C.H. "Fibronectin regulates gene expression and progressive differentiation of osteoblasts" (Abstract). J. Bone Mineral Research, vol. 10, suppl. 1, S273 (1995).

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Heart Rate Dynamics During Microgravity Exposure: Data Analysis

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Funding:

Project Identification: 199-14-11-21

Solicitation: 93-OLMSA-07

Initial Funding Date:

Expiration:

FY 1995 Funding: $99,660

Students Funded Under Research: 0

Responsible NASA Center: Johnson Space Center

Task Description:

NASA has 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. NASA has also prioritized the development of new, efficient and inexpensive ways to store and disseminate the vast amounts of physiological data obtained during space flight and also during ground-based simulations of microgravity exposure.

Our objectives are intended to extend our results developed during our current NASA grant: 1) to compile digitized databases (pre-flight, during flight and post-flight) of continuous ECG recordings from de-identified crew members from U.S. and Mir missions and to store these databases on compact discs (CD-ROM format) to facilitate distribution and retrieval; 2) to correlate the 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; 3) to quantitate the loss of complex heart rate variability as a potentially useful index of cardiac deconditioning during space flight, and during microgravity simulations with bed rest in order to assess the effects of countermeasures such as LBNP and exercise, and 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.

Both bed rest deconditioning and space flight are postulated to alter neuroautonomic function. To gain insight into these perturbations, we analyzed heart rate dynamics using conventional statistics and spectral analysis as well as time series methods derived from nonlinear dynamics ("chaos theory") (1). We studied continuous heart rate data from digitized Holter recordings in 8 healthy female volunteers (age range 28-34 years) who underwent a 13-day, 6-degree head-down bed rest study with serial lower body negative pressure (LBNP) trials (2). Heart rate variability was measured on 5 min. data sets using conventional statistics as well as with a new nonlinear measure of "complexity," termed approximate

entropy (ApEn) (3). Tolerance to LBNP was significantly reduced on bed rest days 4 and 9 (or 11) vs. pre-bedrest. We also found that bed rest caused a significant decrease in the complexity of heart rate variations during LBNP by day 4, consistent with neuroautonomic alterations (1). Measurements of heart rate complexity, using a method derived from nonlinear dynamics, may provide a sensitive marker of this loss of physiological variability, complementing conventional time and frequency domain measures.

We also recently analyzed instantaneous heart rate dynamics in 6 male cosmonauts ages 38-50 yrs in conjunction with Mir missions 6, 7, and 8 (4). Beginning early in-flight there was a significant decrease in heart rate variability associated with a reduction in relatively low frequency (0.1-0.15 Hz) fluctuations. These subtle changes in relatively low heart rate frequency power may be related to altered baroreflex function. However, a variety of measures of short and longer term heart rate variability were surprisingly stable despite the stresses associated with prolonged microgravity exposure aboard the Mir space station. Prominent interindividual variations were noted. Overall, these findings are evidence for the preserved integrity of the feedback systems regulating heart rate dynamics. The vigorous in-flight exercise regimens and use of other measures designed to counter the effects of microgravity and deconditioning may have influenced neuroautonomic responsiveness, although controlled observations were not available.

During the past 2 years, we also produced the first prototype CD-ROM with heart rate data from the Mir Study as well as US astronaut data and bed rest studies, including software utilities to permit investigators to analyze these complex signals. We wish to emphasize that this CD-ROM is more than a mere compilation of very large amounts of interesting physiologic data. Although these recordings were gathered by NASA and by the Soviet space agency at enormous cost, the research value of such a collection would be greatly diminished if the means to analyze it were lacking. This point is best illustrated by NASA's past experience with storage of similar data gathered during the Mercury, Gemini, and Apollo programs; without suitable analytic tools, these irreplaceable recordings were discarded because the cost of storing them was judged to exceed their value. The software contained on this CD-ROM is an essential component. It provides the necessary technology for researchers to study these unique recordings: tools we have developed, debugged, and refined over many years based on our own use of them as well as input from many of our colleagues worldwide who use them daily in their own research.

The results of these above studies have important implications for the design of future terrestrial bed rest and space flight protocols. Additional long-term monitoring of cardiovascular dynamics is particularly needed during episodes of space sickness (5), and with exposure to lower body negative pressure and other orthostatic challenges, postural shifts, and selected pharmacologic agents (6). Investigations are also needed to determine whether interindividual differences in heart rate dynamics can be used to predict successful adaptations to flight or postflight deconditioning. International collaborative investigations aimed at further testing the stability of physiologic control mechanisms during long-duration space flight will be helpful in preparing for future space station development.

Publications, Presentations, and Other Accomplishments:

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Exercise Within LBNP to Produce Artificial Gravity

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Task Description:

Calculations suggest that, to date, exercise in space 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 against 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, 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 long-term bed rest.

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.

Larger waist seal area decreases the LBNP required to produce a given level of footward force. We previously reported that about 100 mm Hg lower body negative pressure (LBNP) is necessary to generate one body weight (BW) of footward force when the negative pressure acts only through the cross-sectional area of the subject's waist. By expanding this area through which the pressure produces force, we hypothesized that the amount of negative pressure required to generate one BW could be decreased. To expand this area, a flexible neoprene waist seal was attached at its outer edge to the LBNP chamber, such that part of the force created by suction acting on the seal was distributed on the chamber, and part was applied to the subject. The area spanned by the flexible seal between the subject and LBNP chamber can be varied by increasing or decreasing the dimensions of the elliptical opening through which the subject placed their lower body into the chamber. In nine supine subjects, we found that if the waist seal area equaled twice the subject's waist cross-sectional area, the slope of the footward force/negative pressure relationship doubled, such that the negative pressure necessary to produce one BW decreased from about 100 mm Hg to 50-55 mm Hg. The reduced negative pressure required to generate one BW of force lowers the risk of syncope, hernia, and petechiae associated with higher levels of LBNP, and creates a more Earth-like ratio of cardiovascular to musculoskeletal stress. Also, shoulder straps attached to the waist seal permit application of part of the suction force on the waist seal to the upper body, thus allowing spinal as well as lower-body loading.

Treadmill exercise while supine in LBNP simulates most aspects of treadmill exercise while upright. Previously, we found that supine exercise during 100 mm Hg LBNP produced equivalent ground-reaction forces but increased heart rates 22% compared to upright exercise in 1 g. We used the larger waist seal during supine LBNP exercise to reduce the systemic cardiovascular stress and to maintain ground-reaction forces equivalent to upright exercise. Eight healthy volunteers ran upright on a horizontal treadmill without LBNP. They also ran supine on a vertical treadmill within a LBNP chamber at 55 ± 3 mm Hg. The average heart rate during upright treadmill exercise (161 ± 4 bpm) was similar to that for exercise during supine LBNP (162 ± 4 bpm). Increasing the waist seal area to twice the subject's waist cross-sectional area during supine exercise provided equivalent cardiovascular response as upright exercise in 1 g.

We hypothesized that oxygen consumption (VO_2) and heart rate during supine treadmill exercise in LBNP equal those during upright treadmill exercise, regardless of treadmill grade. Eight healthy subjects walked (4.5 ± 0.3 km/h, mean ± SD) and ran (8.0 ± 1.0 km/h) at three treadmill angles (downhill -4°, level (0°), and uphill +4°) under both conditions (supine LBNP and upright). Fifty-two ± 4 mm Hg LBNP generated 1 BW of footward force. Subjects exercised for 5 min at each of the 12 posture/gait/treadmill grade conditions. With the treadmill at 0° grade, VO_2 during supine LBNP treadmill exercise (walking: 14.6 ± 0.9 ml x min^{-1} x kg^{-1}, mean ± SE; running: 32.2 ± 1.6) did not differ from that during upright treadmill exercise (walking: 15.1 ± 0.9; running: 34.0 ± 1.9). Increasing treadmill grade from -4° to +4° increased upright running VO_2 by 15.6 ± 1.0 ml x min^{-1} x kg^{-1}, yet the same increase in grade in supine LBNP running only increased VO_2 by 2.8 ± 1.4 ml x min^{-1} x kg^{-1}. Similarly, increasing grade from -4° to +4° increased upright walking VO_2 by 10.2 ± 0.8 ml x min^{-1} x kg^{-1}, yet did not affect VO_2 in supine LBNP walking (D = 0.6 ± 0.5 ml x min^{-1} x kg^{-1}, NS). Heart rate results mirrored VO_2 results. Fundamental differences exist between upright exercise and supine LBNP exercise which discount the use of grade to alter workload during LBNP exercise. Nevertheless, LBNP exercise mimics VO_2 and heart rate responses to treadmill-level upright exercise and therefore, may be useful to simulate gravity for exercise during space flight.

We also hypothesized that walking and running biomechanics during supine LBNP treadmill exercise would duplicate upright walking and running exercise. Eight healthy subjects were filmed while walking and running at self-selected speeds and at treadmill grades of +4, 0 and -4% during both supine

LBNP and upright conditions. During supine LBNP exercise, a subject's legs were suspended from each other via cuffs, bungee cords, and pulleys, such that each leg acted as a counterweight to the other leg during the gait cycle. Maximum rise distance of the foot was higher during upright gait (0.10 ± .004 m) than during gait in the LBNP chamber (0.08 ± 0.006 m). Step frequency was slightly faster in LBNP (1.34 ± 0.02 Hz) than in upright running (1.29 ± 0.02 Hz). Knee and hip flexion during swing phase were somewhat less in LBNP (67 ± 2° and 25 ± 1°, respectively) than upright gait (79 ± 2° and 28 ± 1°, respectively). Footward forces integrated over each stride were not significantly different between LBNP and upright exercise. Force generation during gait is a known factor for maintaining bone density in 1G. The subtle kinematic differences between supine LBNP and upright treadmill exercise are likely due to the leg suspension system and horizontal orientation of the subject in gravity, and would thus be eliminated during space flight.

Self-generated LBNP exercise permits aerobic and resistance training with no external power. Allowing the legs themselves to generate the negative pressure against which they work is a simple, inexpensive, and compact way to accomplish LBNP exercise without an external power source. A self-generated LBNP device consists of a flexible cylinder, sealed around the lower body, which expands and collapses longitudinally, but not radially. As the legs push footward, the cylinder expands, decreasing internal air pressure, and increasing the generated footward force. Negative pressure is limited by an adjustable valve to control air flow into the chamber. Force depends on air inflow rate, cylinder volume, and rate of expansion. We hypothesized that this device could be used to generate substantial footward forces and provide simultaneous cardiovascular stress. Seven healthy subjects performed supine knee bend exercise in the self-generated LBNP device for 5 to 6 min. Exercise rate was maintained at 20 cycles/min and the inflow valve was adjusted so footward force during cylinder expansion peaked at approximately 150% of body weight. Maximum footward force at the peak of the exercise cycle averaged 1116 ± 87 N (114 ± 9 kg), and pressure within the cylinder concomitantly decreased 26 ± 3 mm Hg below ambient. Heart rate and oxygen consumption increased 75 ± 4 beats/min and 26.3 ±14 ml O_2/kg/min from supine resting values, respectively. In addition, two supine subjects performed maximal efforts with the inflow valve completely closed, and achieved 332% and 337% of body weight equivalent force and concomitant pressure decreases of 63 mm Hg and 62 mm Hg, respectively. Depending on the setting of the inflow valve, this device can emphasize cardiovascular (rapid, low resistance) or musculoskeletal (slow, high resistance) conditioning. Exercise with self-generated LBNP may provide a low cost, low mass countermeasure to musculoskeletal and cardiovascular deconditioning in space while minimizing exercise time and payload disturbance. For this work, Richard Ballard was a finalist in the student award competition at the 1993 Aerospace Medical Association meeting. Don Watenpaugh received US patent # 5,356,361 for the self-generated oscillating pressure exercise concept in October, 1994, and he received a Patent Award from NASA Ames Commercial Technology Office in 1995.

LBNP vs. centrifugation to simulate cardiovascular effects of gravity. Gravity creates blood pressure gradients which redistribute body fluids towards the feet and elicit lower body vasoconstriction. We hypothesized that artificial orthostatic stresses such as Gz centrifugation and LBNP differ from whole-body tilting (normal gravitational stress) in terms of the distribution of microvascular blood flow. Cutaneous microvascular flows were measured by laser Doppler flowmetry at the neck, thigh, and leg of 15 normal subjects. Volunteers underwent stepwise head-up tilt (HUT) and short- and long-arm centrifugation protocols from supine control (0 Gz) to 0.2, 0.4, 0.6, 0.8, 1.0, 0.8, 0.6, 0.4, 0.2, and 0 Gz at the feet, for 30 s periods with 10 s transitions between levels. The same subjects underwent a corresponding supine LBNP protocol, up to 100 mm Hg (in 20 mm Hg increments) and back to zero pressure, which produced transmural pressure across blood vessels in the foot approximately equal to the HUT protocol. In general, application of all orthostatic stresses produced significant flow reductions in the lower body. At low levels of each stress (0.4 Gz, 40 mm Hg), LBNP generated the greatest relative reduction in flow in the lower body (-66.9 ± 5.7%, thigh; -60.6 ± 5.7%, leg, mean±SE). HUT caused a less severe flow reduction than LBNP at the thigh and leg (-39.9 ± 8.1% and -55.9 ± 4.8%), while the effects induced by both forms of centrifugation were the least profound. Higher levels of each stress generally resulted in similar responses. Therefore, in terms of lower body

vasoconstrictor responses, LBNP produces more cardiovascular stress than normal gravity, whereas centrifugation produces less.

We further hypothesized that the magnitude of upper-to-lower body fluid redistribution would increase according to the following order: short-arm centrifugation (SAC), long-arm centrifugation (LAC), head-up tilt (HUT), and LBNP. We employed strain gauge plethysmography of the neck, thigh and calf during the HUT, centrifugation, and LBNP protocols described above. Control measurements were made while supine. SAC and LAC elicited similar increases in thigh volume at 1Gz (2.3 ± 0.4 and $2.1 \pm 0.1\%$, respectively, $n > 7$). At 100 mm Hg LBNP, thigh volume increased ($3.4 \pm 0.3\%$) significantly more than during 1Gz centrifugation ($p < 0.05$). Surprisingly, due to a paradoxical 0.6% reduction of thigh volume between 0.8 and 1.0 Gz HUT, thigh volume was increased only $0.6 \pm 0.3\%$ at 1Gz HUT. The calf demonstrated similar, although less definitive, responses to the various gravitational stimuli. Neck volume decreased less during HUT than during the other stimuli. Heart rate increased similarly during HUT (18 ± 2 beats/min) and LAC (12 ± 2 beats/min), and exhibited still greater elevation during LBNP (29 ± 4 beats/min), yet did not increase during SAC. These results suggest upright posture activates mechanisms that counteract footward fluid redistribution which are not activated during supine applications of simulated gravity. In terms of fluid redistribution and heart rate, LAC more closely approximated effects of normal gravity (HUT) than LBNP. Therefore, when considering LBNP to simulate gravity, these findings support efforts to reduce the cardiovascular stress imposed by LBNP, while preserving the gravity-like force generated by LBNP. For this work, Donald Watenpaugh received the First Place Student Award in Animal Physiology at the 1994 Annual Meeting of the American Society for Gravitational and Space Biology.

Daily supine LBNP exercise protects subjects from physiologic deconditioning during five days of bed rest. Integrated physiologic countermeasures are needed to maintain orthostatic tolerance after space flight or bed rest. We hypothesized that supine exercise during LBNP would prevent bed rest-induced loss of orthostatic tolerance by preventing hemoconcentration. Fifteen male subjects underwent 5 days of 6° head-down bed rest: 5 control subjects did not exercise, and 10 performed 30 min/day of supine interval treadmill exercise at intensities up to 90% VO_2 peak. One body weight of footward force was generated by 55 ± 3 mm Hg LBNP during supine exercise on a vertical treadmill. Pre- and post-bed rest orthostatic tolerance was assessed as time to presyncope during 80° head-up tilt (30 min max). Mean head-up tilt tolerance was unchanged in the subjects who performed 30 min/day LBNP exercise during bed rest (pre: 25.9 ± 2.8 min, $X \pm SE$; post: 28.2 ± 1.8 min; NSD). In contrast, tilt tolerance time in non-exercising control subjects decreased from 27.3 ± 2.7 min to 22.4 ± 4.0 min. Hematocrit increased from 41.8 ± 1.1 to $45.0 \pm 1.0\%$ in the control group during 5 days of bed rest, indicating substantial hemoconcentration. Hematocrit did not increase significantly in the group performing LBNP exercise (42.8 ± 0.8 vs. $43.5 \pm 0.8\%$; NSD). The two groups exhibited similar mean heart rates and arterial blood pressures during orthostasis after bed rest. These results indicate that LBNP exercise during bed rest prevents hemoconcentration, which in turn, helps maintain orthostatic tolerance during bed rest. For this study, Donald Watenpaugh received the Proctor and Gamble Graduate Student Award at the Experimental Biology 1994 meeting.

We also hypothesized that daily supine exercise with LBNP would be as effective as upright exercise in maintaining upright exercise responses after 5 days of 6° head-down bed rest. Twenty-four healthy men were randomly assigned to one of three groups ($n = 8$ per group). The control group did not exercise, the upright group performed a daily, 30 min interval exercise protocol on a treadmill, and the LBNP exercise group performed the same exercise protocol while supine, as described above. All subjects performed a graded upright treadmill test before and immediately after bed rest at three exercise levels. After bed rest, only the control group exhibited significant increases in heart rate (176 ± 3 pre-bed rest, 185 ± 2 post), respiratory exchange ratio (1.03 ± 0.02 pre-bed rest, 1.12 ± 0.03 post), and ventilation (90 ± 5 l x min^{-1} pre-bed rest, 103 ± 5 post) by treadmill exercise level three ($VO_2 = 41 \pm 1$ ml x min^{-1} x kg^{-1}). These results indicate that supine exercise with LBNP is as effective as upright exercise in maintaining upright exercise responses during bed rest, and should be considered as a possible countermeasure to help sustain egress capability after space flight.

Taken together, the above results strongly support continued development of LBNP exercise as a cost-effective alternative to centrifugation for periodic simulation of gravity during long-term existence in microgravity. Thus, our current efforts focus on completion of a longer, 2-week bed rest study with a more comprehensive battery of pre- and post-bed rest tests to evaluate more fully the efficacy of LBNP exercise for prevention of bed rest-induced physiologic deconditioning. Even longer duration studies may be necessary to evaluate treadmill exercise within LBNP as a countermeasure for maintaining calcium homeostasis and bone/cartilage strength and function.

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.

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Neural Control Mechanisms and Body Fluid Homeostasis

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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 of the brain 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 monoamine 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.

Traditionally, one of the complications in identifying afferent pathways from systemic receptors that sense decreases in body fluids is that cutting the cervical vagus to remove afferent nerves also destroys vagal efferents. Destroying vagal efferents induces debilitation and a severely compromised preparation. A recently developed technique permits selective removal of vagal afferents while leaving efferent fibers intact. This method, which will be a mainstay in our experimental approach to study the role of vagal afferents, involves the application of kainic acid to the nodose ganglia. Because of the pivotal nature of this technique, we have performed dose and validation studies to more thoroughly characterize the effects of kainic acid applied to the nodose ganglia. In these experiments, kainic acid was applied to either the right or left nodose ganglia (doses 0.8-2.0 (g). The opposite ganglion was injected with isotonic saline as control. Five rats studied were sacrificed two weeks later for examination of the ganglia. Quantitation indicated that kainic acid administered at a dose of 2g reduced the number of cell bodies by 80% in the treated sides as compared to the control ganglia.

Because kainic acid administration does not completely eliminate the cell bodies in the nodose, a functional test of the remaining cells was performed. Active transport of the dye, True Blue, from nerve terminals to the chest or abdominal cavities to cell bodies in the nodose was used as a means to test the viability of cells. True Blue dye is actively taken up by nerve endings and transported to cell bodies in healthy cells. For instance, microscopy allows visualization of True Blue in the ganglia. True Blue is not taken up by nerve endings of dying or dead cells and subsequently does not appear in the cell body. In these experiments, nodose ganglia-kainic acid treated rats (n=5) were injected with 5% True Blue in either end of the chest or abdominal cavities. Seven days later, the rats were sacrificed and the nodose ganglia examined. From these experiments, it was found that there was approximately a 90% reduction in the number of viable cells in ganglia treated with neurotoxin.

As a further functional test of vagal deafferentation, we have employed the Bezold-Jarisch reflex which has been classically used to characterize removal of cardiopulmonary afferents immediately after vagotomy. One component of the Bezold-Jarisch reflex depends upon activation of low pressure baroreceptors in the cardiopulmonary circulation. When this component of the reflex is absent, the central nervous system does not receive input from low pressure baroreceptors. Therefore, in another series of experiments, we studied the Bezold-Jarisch reflex in nodose ganglia-kainic acid treated (2 g to both nodose ganglia) rats and control rats. Bezold-Jarisch reflexes were accessed by examining the reductions in mean arterial pressure (AP) and heart rate (HR) in response to the intravenous administration of serotonin (5-HT). The rats were studied 10 days after kainic acid treatment when they were vigorous and healthy. Baseline AP and HR were equal between groups. However, kainic acid treated animals were significantly attenuated in their AP and HR responses to each dose of 5-HT. These data clearly indicate that kainic acid treatment severely compromises the function of vagal afferents which include, most notably for our studies, those derived from low pressure cardiopulmonary receptors.

In previous work, we observed that removal of high pressure arterial baroreceptor input by performing sinoaortic baroreceptor deafferentation impairs the ingestion of sodium chloride solutions in response to reductions in body sodium and hypovolemia. We have recently attempted to determine whether removal of specific arterial baroreceptor input would have the same effect. That is, the present experiment was designed to determine whether removal of carotid sinus afferents vs. aortic arch

baroreceptors vs. both sets of receptors was critical for producing deficits in sodium solution ingestion. Rats maintained on standard rat chow, water and 0.3 M NaCl underwent surgery for transection of aortic depressor nerve (ADN), the carotid sinus nerve (CSN) or both (i.e., sinoaortic baroreceptor deafferentation; SAD). SAD involves loss of input from the superior laryngeal nerve (SLN) which does not contain baroreceptors so an additional group of control animals underwent transection of the SLN. Sham operated rats underwent identical surgical procedures except no nerves were transected. Four weeks after surgery, all rats received two injections of furosemide (10 mg/kg, sc) 30 minutes apart which induces extracellular fluid depletion through its diuretic and natriuretic action. Animals were returned to their home cage which contained access to distilled water and a sodium deficient diet. The next morning access to 0.3 M NaCl and water was provided and intakes of the fluid recorded every 30 minutes over the next two hours. The results of the experiment indicate that there was a significant effect of surgical condition X time interaction. SAD rats drank significantly less sodium in the early stages of sodium access compared to all other groups. There was no significant difference in saline ingestion between the other groups. There were no effects on water intake. The results indicate that removal of either the ADN or CSN baroreceptors alone are not sufficient to produce reductions in saline solution ingestion but the complete removal of arterial baroreceptor input, that is, SAD is necessary. This experiment provides further important information about the afferent pathways used to maintain body fluid homeostasis.

Past space flights have indicated that severe dehydration is a common problem in microgravity. Microgravity-induced dehydration has been suggested to be due to various causes such as: 1) the position of astronauts during launch; 2) cephalad redistribution fluids due to a lack of gravity; 3) altered hormonal secretions (i.e., reduced VP and renin; increased natriuretic hormone); 4) nausea; 5) medication, and 6) reduced thirst. The consequences of prolonged dehydration and reduction of extracellular/blood volume has been suggested to be a major factor contributing to the severe orthostatic intolerance that commonly occurs in astronauts upon returning to earth.

It has been hypothesized that a centralization of blood volume stimulates thoracic baroreceptors which in turn activate reflex responses (i.e., both neural and efferent hormonal changes) that increase renal excretion of sodium and water. Reasoning by analogy, it is plausible that reductions in the motivation to drink (i.e., impaired thirst) which occurs in the microgravity environment may also entail blood volume shifts and/or baroreceptor stimulation which in turn inhibit behaviors associated with fluid intake. However, because of a lack of basic knowledge about the systemic receptor systems and afferent pathways that mediate thirst, such an interpretation must remain conjuncture.

Under conditions in which the CNS 1) receives inappropriate input from one or more afferent sensory sources , or 2) incorrectly processes this input, there will be erroneous output to the effectors responsible for determining fluid homeostasis. Because of the deleterious consequences arising from disordered information processing within the afferent systems and neural network regulating body fluid balance, it is critical to understand the 1) nature and locus of receptor systems; 2) mechanisms of coding afferent signals; 3) extent of the central processing system; 4) neurophysiological and neurochemical mechanisms employed in information processing within each of the components of the visceral neuraxis, and 5) nature of neural plasticity that produces long-term adaptation of these systems in the face of altered afferent input. Any or all of these mechanisms may contribute to microgravity-induced dehydration and its consequences such as orthostatic intolerance. A thorough understanding of the CNS role in the regulation of body fluid homeostasis may lead to the development of effective countermeasures. As an example, in the course of our work on basic mechanisms of thirst and sodium appetite, we have recently discovered that both systemically and centrally administered yohimbine, an 2-adrenergic receptor antagonist, induces a remarkable sodium appetite which is accompanied by increased thirst. Therefore, yohimbine which has been explored as a potential therapeutic agent for autonomic insufficiency may facilitate behaviors and reflexes which expand extracellular blood volume. A better understanding of the role of central and systemic mechanisms in the control of body fluid homeostasis will lead to the development of rational pharmacological countermeasures to reduce dehydration and orthostatic intolerance in the microgravity environment.

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.

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Validation of Spectral Analysis as a Noninvasive Tool to Assess Autonomic Efferent Regulation of Cardiovascular Function

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Expiration: 5/96
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Students Funded Under Research: 15

Task Description:

A major focus of our program is to develop a sensitive noninvasive procedure to identify the autonomic components of human cardiovascular regulation during hypo/hypertensive challenges. In this second year, 10 women (in the first year, 10 men) volunteers were tested to determine changes in the sympatho-vagal balance of autonomic control of cardiovascular regulation during graded headward and footward blood volume shifts. Autonomic blockade was used to unmask the relative contribution of sympathetic and parasympathetic efferent influences in response to 10 min each of 20 and 40 mmHg lower body negative (LBNP) and 15 and 30 mmHg positive (LBPP) pressure. Mean values of humoral variables [hematocrit (HCT), catecholamines (NE and EPI), plasma renin activity (PRA), and pancreatic polypeptide (PPP)] and mean and spectral analysis of R-R interval (HR), arterial pressure (AP), skin (SF) and radial artery (RF) blood flow, stroke volume (SV) and total peripheral resistance (TPR) were determined for all phases of the study. The primary objective of the study was to indicate which changes in mean values and/or spectra of cardiovascular variables consistently correlated with changes in sympatho-vagal balance in response to headward and footward fluid shifts. A secondary objective was a comparison of the responses of healthy men and women volunteers to autonomic blockade and lower body pressure stresses. The principal hypothesis being tested was that headward fluid shifts would evoke an increase in parasympathetic activity and footward fluid shifts would evoke an increase in sympathetic activity both of which could be detected by spectral analysis and verified by hormonal changes.

Hormonal indices demonstrated that men had less relative plasma volume (higher HCT) than did women. Indices of both sympathetic (NE and EPI) and parasympathetic (PPP) activity were also greater in men than women. The response of both sexes to LBNP was to increase catecholamines, but the increase was greater in men than women. Men responded to LBPP with an increase in PPP. Beta blockade increased EPI more in men than women and muscarinic blockade decreased PPP in men but not in women.

Hemodynamic indices demonstrated that men had higher mean AP and TPR, lower HR and no difference in SV from women. Muscarinic blockade increased AP, HR and TPR in both groups and beta blockade increased TPR in women but not in men. During LBNP, unblocked AP was controlled in a similar manner in both groups, AP was maintained by increases in HR and TPR that countered decreases in SV. After muscarinic blockade, the decrease in SV and the increase in TPR were greater, after beta blockade, the decrease in SV and the increase in TPR were smaller. During LBPP, unblocked AP was maintained by slight decreases in SV and HR that countered increased TPR. After muscarinic blockade, the increase in TPR was greater.

When spectral power indices from women were compared with those from men, men had greater overall power for all variables in all frequency bins [low (LF), 0.006 to 0.05 Hz; mid (MF), 0.05 to 0.15 Hz; high (HF), 0.15 to 0.45 Hz]. However, the relative amounts of power in MF and HF bins were greater in SV, CO and TPR for women than for men. Women had less distribution of HR and CO power in the LF bin than did men. Beta blockade increased MF and HF power of HR and TPR in women, but not in men. Muscarinic blockade almost ablated all HR power and had no effect on TPR power in both men and women.

In summary, these studies demonstrated that men had higher resting values of circulating hormones that are markers for sympathetic and parasympathetic activity and lower relative plasma volume. The hemodynamic consequences of the greater concentrations of vasoactive agents were greater resting unblocked mean TPR and greater spectral power of TPR in men. Since beta blockade led to an increase in TPR in women but not in men, we conclude that women had greater tonic vasodilation than did men. This observation was verified by a significant increase in TPR spectral power in response to beta blockade in women but not in men. Similarly, men and women had the same intrinsic HR, but men had significantly lower resting HR indicating increased tonic parasympathetic activity in men. Again, after beta blockade, HR spectral power increased in women but not in men implying that women demonstrated a tonic beta adrenergic component in the regulation of HR.

The data collection has been completed; abstracts will be/have been presented at meetings and manuscripts are being prepared. Results from these studies served as a basis for two proposals: one submitted in response to NASA's April 1995 NRA and one in response to the DOD RFP concerning military women's health.

Direct applications of this study are currently being performed in the Division of Cardiology where Dr. Fabio Leonelli is conducting studies of unexplained syncope in patients referred from area physicians. To date 11 patients with a diagnosis of unexplained syncope and 8 controls have been tested in a tilt test protocol using an experimental team, equipment, spectral analysis techniques and hormonal assays developed under this NASA protocol.

Dr. Leonelli's clinical staff are also directly involved in these studies as are the staff of the University's General Clinical Research Center. Our research group is committed to participate in the development of a syncope clinic at the University of Kentucky in which procedures found to be effective in diagnosing impending syncope in normal subjects would be applied to patients with unexplained syncope.

Publications, Presentations, and Other Accomplishments:

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Adaptation in Artificial Gravity Environments

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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 1g underestimate or overestimate the effects to be expected during rotation in environments with a background force level less than 1g, 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, c) 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.

We have completed a series of studies demonstrating that both arm and head movement control can be adapted to high velocities of rotation in an artificial gravity environment by making large numbers of head movements at increasing dwell velocities. Adaptation can take place in the absence of visual feedback about performance solely on the basis of proprioceptive, somatosensory, and motor command monitoring. The rate of adaptation is increased, however, by approximately 40% if vision is permitted. We have found, too, that for a given velocity of vehicle rotation adaptation occurs at a slower rate if the gravitoinertial resultant force is increased by as small an amount as 0.1g. This finding points to alterations in muscle spindle gain through otolith spinal effects influencing the adaptive process. We have studied an individual lacking proprioceptive function below the neck and found no evidence of adaptation to Coriolis force perturbations of limb trajectory. All of our studies point to the critical role of proprioception and somatosensation in adaptation to artificial gravity environments. This year we will be evaluating whether adaptation to rotation in a 1g force background transfers to 0g and 1.8 force backgrounds in parabolic flight. Our studies to date point to rotation as a feasible way of providing artificial gravity in long duration missions.

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.

Publications, Presentations, and Other Accomplishments:

Cohn, J., P.DiZio, and J.R.Lackner "Reaching errors are made to stationary targets presented in full field moving virtual environments (VE)." ARVO Annual Meeting, 1995.

DiZio, P., and J.R.Lackner "Head loading affects perceived orientation during body rotation." Society for Neuroscience Annual Meeting, 239.11, 1994.

DiZio, P . and J.R.Lackner "Effects of Coriolis, cross-coupled stimulation on head movement control." Society for Neuroscience Annual Meeting, 59.6, 1995.

DiZio, P. and J.R.Lackner "Motor adaptation to Coriolis force perturbations of reaching movements: Endpoint but not trajectory adaptation transfers to the non-exposed arm." J. Neurophysiology, 74(4), 1787-1792 (1995).

DiZio, P. and J.R.Lackner Inertial Coriolis force perturbations of arm and head movements reveal common, non-vestibular mechanisms. "Multisensory control of posture." Edited by: Mergner, T. and F. Hlavacka Plenum Press, NY, pp 331-338, 1995.

Jeka, J. and J.R. Lackner "The role of haptic cues from rough and slippery surfaces on human postural control." Experimental Brain Research, 103, 267-276 (1995).

Jeka, J.J. and J.R. Lackner Fingertip touch as an orientation reference for human postural control. "Multisensory control of posture." Edited by: Mergner, T. and F. Hlavacka. Plenum Press, NY, pp 213-221, 1995.

Jeka, J.J., G. Schoner, J.R.Lackner "Entrainment of postural sway to sinusoidal haptic cues." Society for Neuroscience Annual Meeting, 146:14, 1994.

Lackner, J.R. " Proprioceptive contributions to limb movement control." Society for Neuroscience Abstracts:Symposium, New Vistas in the Control of Arm Movement Trajectories 300.5, (1995).

Lackner, J.R. and D. Collins "The United States Air Force spatial orientation program." J. of Vestibular Res., 5, (1995).

Lackner, J.R. and P. DiZio. "Rapid adaptation to Coriolis force perturbations of arm trajectory." J. Neurophysiol., 72(1), 299-313 (1994).

Lackner, J.R. and P.DiZio. "Generalization of adaptation to Coriolis force perturbations of reaching movements." Society for Neuroscience Annual Meeting, 756.10, 1995.

Lackner, J.R., R. Easton, E.Bentzen, P.DiZio "Adaptation to Coriolis force perturbations of reaching movements in the blind." Society for Neuroscience Annual Meeting, 490.16, 1994.

Motor Adaptation to Coriolis and Contact Forces

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Funding:

Project Identification: 199-16-17-05 Solicitation: 93-OLMSA-07

Initial Funding Date: 2/94 Expiration: 1/97

FY 1995 Funding: $196,482 Students Funded Under Research: 8

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.

Reaching movements made in a rotating artificial gravity environment are initially deviated both in trajectory and endpoint by the inertial Coriolis forces generated by the forward velocity of the arm. We found earlier that with repeated movements adaptation occurs even in the absence of visual feedback. We have now found that the nature of the adaptation depends on whether terminal contact of the hand with a surface occurs at the end of the movement. In the absence of contact, individuals acquire trajectory adaptation but not endpoint adaptation--they again reach in straight paths, but to the wrong place. With contact, they adapt completely, both trajectory and endpoint. If one arm is used during exposure to Coriolis forces (and terminal contact of the hand is allowed), then limited intermanual transfer of adaptation occurs. Endpoint adaptation but not trajectory adaptation transfers. The independent representation of movement trajectory and endpoint shown by these studies is inconsistent with current equilibrium point models of movement control and emphasizes that the nervous system likely uses a forward model in movement implementation. These studies showed us the tremendous importance of somatosensory feedback and proprioception in adaptation and led us to ask whether such feedback would also be useful in postural and orientation control. We conducted several studies involving delicate touch of the index finger with a stable surface during stance. Such contact greatly enhances postural stability in normal, blind, and labyrinthine defective subjects. These findings are

enabling us to design procedures for enhancing postural and locomotor stability in rotating environments.

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. These studies provide new avenues for developing rehabilitation and training programs for individuals with loss of labyrinthine function and other types of balance disorders. We are also exploring the use of such contact cues in minimizing sensory-motor re-entry disturbances in astronauts following space flight.

Publications, Presentations, and Other Accomplishments:

Cohn, J., P. DiZio, and J.R.Lackner "Reaching errors are made to stationary tartgets presented in full field moving virtual environments (VE)." ARVO Annual Meeting, 1995.

DiZio, P., and J.R.Lackner "Head loading affects perceived orientation during body rotation." Society for Neuroscience Annual Meeting, 239.11, 1994.

DiZio, P., and J.R.Lackner "Motor adaptation to Coriolis force perturbations of reaching movements: Endpoint but not trajectory adaptation transfers to the non-exposed arm." J. Neurophysiology, 74(4), 1787-1792 (1995).

DiZio, P. and J.R.Lackner Inertial Coriolis force perturbations of arma nd head movements reveal common, non-vestibular mechanisms. "Multisensory control of posture." Edited by: Mergner, T. and F. Hlavacka. Plenum Press, NY, pp 331-338, 1995.

DiZio, P., R.J. Lackner "Effects of Coriolis, cross-coupled stimulation on head movement control." Society for Neuroscience Annual Meeting, 59.6, 1995.

Jeka, J., and J.R.Lackner "The role of haptic cues from rough and slippery surfaces on human postural control." Experimental Brain Research, 103, 267-276 (1995).

Jeka, J.J. and J.R. Lackner Fingertip touch as an orientation reference for human postural control. "Multisensory control of posture." Edited by: Mergner T. and F. Hlavacka. Plenum Press, NY, pp 213-221, 1995.

Jeka, J.J., G. Schoner, J.R. Lackner "Entrainment of postural sway to sinusoidal haptic cues." Society for Neuroscience Annual Meeting, 146.14, 1994.

Lackner, J.R. "Proprioceptive contributions to limb movement control." Society for Neuroscience Abstracts: Symposium, New Vistas in the Control of Arm Movement Trajectories. 300.5, (1995).

Lackner, J.R. and D.Collins T"he United States Air Force spatial orientation program." J. of Vestibular Res., 5, (1995).

Lackner, J.R. and P.DiZio "Rapid adaptation to Coriolis force perturbations of arm trajectory." J. Neurophysiol., 72(1), 299-313 (1994).

Lackner, J.R. and P. DiZio. "Generalization of adaptation to Coriolis force perturbations of reaching movements." Society for Neuroscience Annual Meeting, 756.10, 1995.

Lackner, J.R., R. Easton, E. Bentzen, P. DiZio. "Adaptation to Coriolis force perturbations of reaching movements in the blind." Society for Neuroscience Annual Meeting, 490.16, 1994.

Carbon Dioxide-Oxygen Interactions in Extension of Tolerance to Acute Hypoxia

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Funding:

Project Identification: 199-14-17-14
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1995 Funding: $120,000
Students Funded Under Research: 3

Task Description:

Studies in this and other laboratories have shown clear improvement in useful consciousness of normal men at rest when atmospheric CO_2 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 deadaptation to hypoxia, producible by control of inspired CO_2. NASA relevance is to accidental or intentional exposure to hypoxic atmospheres in any aspect of present or long-range manned space activity.

Prior year research determined effects of acute atmospheric hypoxia (0.12 and 0.10 ATA O2) in rest and sequential exercise at 50 and 100 W upon arterial hemoglobin O_2 saturation, pulmonary ventilation and alveolar gas composition, heart rate, and selected mental functions. The purpose of 30 and 60 minute acute exposures to hypoxia alone was to establish baselines for quantitating degree and dynamic time courses of carbon dioxide effects responsible for improving tolerance to abrupt inspiratory hypoxia. 0.10 ATA was identified as a level of prominent physiological changes and definitive mental function decrement in stable states, allowing current year investigation of dynamic relations of acute physiological adaptations to hypoxia alone and combined inspiratory hypoxia and hypercapnia.

Progress in the current year has included integration of breath-by-breath and heart beat-by-beat measurements to derive dynamic relations of rates of change of brain blood flow, alveolar gas composition, arterial O_2 saturation and content, and brain O_2 flow. This dynamic data prepares for answering the questions of rates and degrees of changes in brain oxygen partial pressures, and rates of decrement and recovery of mental functions in acute exposures to a range of hypoxic atmospheres. Future work requires modeling of hypoxic adaptation and use of CO_2 to provide acceleration of hypoxic adaptation.

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 spacecraft or submersibles, in aviation and high altitude exposures, in clinical medical emergencies on Earth or in space. Impacts and benefits for the common man of this research and technology relate to improved respiratory support procedures in serious disease, and to safety at work in hazardous closed spaces.

Publications, Presentations, and Other Accomplishments:

Clark, J. M. Modification of oxygen tolerance in rats by adaptation to combined hypoxia and hypercapnia.. Undersea Hyperbaric Med., 21(3), 251-264 (1994).

Clark, J. M., R. Gelfand, C. J. Lambertsen, G. Beck, Jr., and K. R. Hardy. Ventilatory, arterial PCO_2, and cerebral circulatory responses to incremental exercise during O_2 breathing at 2.0 ATA.. Undersea Hyperbaric Med. 22(Supp.), 69, (1995).

Clark, J. M., R. Gelfand, C. J. Lambertsen, W. C. Stevens, G. Beck, Jr., and D. G. Fisher. Human tolerance and physiological responses to exercise while breathing oxygen at 2.0 ATA. Aviat. Space Environ. Med., 66, 336-345 (1995).

Clark, J. M., R. Gelfand, G. Beck, Jr., B. A. Youdelman, E. J. Hopkin, and C. J. Lambertsen. Effects of head-out and total immersion at 1.0 ATA on ventilatory and cerebral circulatory responses to progressive hypercapnia. Abstract. Undersea Hyperbaric Med. 21(Supp.), 36, (1994).

Gelfand, R., and G. Beck, Jr. Transcranial doppler adaptation to monitor MCA blood flow velocity during immersion and dry conditions while exercising and at rest. Undersea Hyperbaric Med, Submitted, (1995).

Gelfand, R., B. A. Youdelman, J. M. Clark, C. J. Lambertsen. Mental performance and physiological effects of hypoxia (0.1 ATA O2 insp.): Effects of restored normocapnia. Abstract. FASEB J., 8(4), A554 (1994).

Gelfand, R., C. J. Lambertsen, G. Beck, Jr., and J. M. Clark. Dynamic responses of SaO_2 and "CBF" to abrupt exposure to inhaled 10% O_2 / 4% CO_2 at rest, followed by 50 and 100 watts exercise. Undersea Hyperbaric Med. 22 (Supp.), 70, (1995).

Gelfand, R., J. M. Clark, G. Beck, Jr., B. A. Youdelman, E. J. Hopkin, and C. J. Lambertsen. Effects of head-out and total immersion on ventilatory and cerebral circulatory responses to progressive exercise at 1.0 ATA in humans. Abstract. Undersea Hyperbaric Med. 21(Supp.), 37, (1994).

Lambertsen, C. J. Acute physiologic adaptations to acute hypoxia. Relation to fire prevention and extinguishment in closed spaces. Abstract. Undersea Hyperbaric Med. 21(Supp.), 38, (1994).

Lambertsen, C. J., and R. Gelfand. Comparison of CO_2-induced improvements in arterial SaO_2 during abrupt exposures of human subjects to .12 and .10 ATA inspired O_2 in N_2, in rest and exercise. Undersea Hyperbaric Med., Submitted, (1995).

Spatially Oriented Database for Digital Brain Images

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Funding:

Project Identification: n/a
Solicitation:

Initial Funding Date: 10/95
Expiration: 6/98

FY 1995 Funding: $187,814
Students Funded Under Research: 2

Joint 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 Baltimore Longitudinal Study on Aging, which follows 180 patients over a 9 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.

Project funding began in October 1995. In that time we have developed extensions to Illustra, an extensible object-relational DBMS, which add a family of datatypes of the form image(X), which are 3D (volumetric) images, e.g. MRI, PET, etc. X is a simple datatype such as Boolean, integer, float, color, vector, or tensor. A datatype such as image (Boolean) can be used to describe regions of interest. Image (integer) or image (float) correspond to conventional image data. Image (color) describes a rendering of an image using a particular coloring scheme. Vector and tensor datatypes relate to the particular coloring scheme. Vector and tensor datatypes relate to the representation of morphological variations, and have not yet been implemented. Image datatypes also have an underlying format, or implementation type, which could in principle be any of a variety of representations of the datatype; only line-segment and array-based representations have been implemented to date. Operations on image datatypes have been added to Illustra and "surfaced" in SQL, including image addition, intersection (multiplication), and coercion between types; e.g. image (integer) can be converted to image (color) for display purposes. A rudimentary Web interface has been developed that allows SQL queries to be posed and the results displayed in a Web browser; queries can return symbolic or image results or a mixture of the two. 3D images must be sliced and converted to GIF in order to be displayed on the Web; slicing and GIF conversion are available as operators on the extended datatypes in SQL.

Statistical operations have also been added to the database, including an implementation of 2 x 2 contingency table Chi Square tests, implemented as a relational aggregator. The database has been loaded with lesion/deficit data from 117 patients who exhibited cortical lesions in MR scans administered as part of the Cardiovascular Health Study protocol. The segmented lesions are represented in the database as image (Boolean) objects. Also several anatomical atlases have been loaded, using image (Boolean) to represent the individual structures, and image (color) to represent the consolidated atlases. Neurological function scores on the CHS patients have also been loaded. Tests for lesion/deficit correlation are performed relative to an atlas structure and neurological variable of interest by forming the contingency table of lesion in structure (yes or no) versus deficit in variable (yes or no); each patient is represented by two Boolean values that place them in a cell in the table, and the chi square aggregation function is applied to the set of patients (Boolean pairs) retrieved by a query to determine the significance of the correlation. The results of this analysis for the initial data set are expected very soon.

This research will provide insights into the localization of brain functions and the effects of stroke on brain function; also 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 datatype-specific operations with general purpose relational ones. When the datatypes are spatially oriented, the result will hopefully be more powerful spatial database technologies.

Altered Brain Vasoregulation in Orthostatic Intolerance

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Funding:

Project Identification: 199-14-17-11
Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95
Expiration: 2/98

FY 1995 Funding: $256,158
Students Funded Under Research: 0

Task Description:

The overall objective 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 are able to simultaneously record cardiovascular indices, EEG, and transcranial Doppler wave form continuously at rest and during tilt-up. We are on schedule. To date we have completed 19 control subjects, 10 patients with neurogenic orthostatic hypotension, and 8 patients with orthostatic tachycardia. Detailed analysis is underway. Preliminary evaluation, based on a comparison of transcranial Doppler and Finapres waveform comparison, in response to tilt and the Valsalva maneuver, demonstrates different patterns of responses in patients, suggesting that brain vasoreactivity may not be concordant with systemic vasoreactivity. We have developed the algorithms to evaluate the effect of tilt on the EEG. We are also proceeding with an evaluation of the value of resistance training in increasing muscle strength, bulk, and reducing venous pooling in patients with orthostatic intolerance.

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 bed rest. 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 countermaneuvers. The studies of the brain, and in particular on 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 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 countermaneuvers and resistance training.

Publications, Presentations, and Other Accomplishments:

Low PA, Opfer-Gehrking TL, McPhee BR, Fealey RD, Benarroch EE, Willner CL, Suarez GA, Proper CJ, Felten JA, Huck CA, Corfits JL "Prospective evaluation of clinical characteristics of orthostatic hypotension." Mayo Clin. Proc., vol. 70, 617-622 (1995).

Low PA, Opfer-Gehrking TL, Textor SC, Benarroch EE, Shen W-K, Schondorf, Suarez GA, Rummans TA "Postural tachycardia syndrome (POTS)." Neurology, Vol. 5, Suppl. 5, S19-S25 (1995).

Low PA, Opfer-Gehrking TL, Textor SC, Schondorf, Suarez GA, Fealey RD, Camilleri M "Comparison of the postural tachycardia syndrome (POTS) with orthostatic hypotension due to autonomic failure." J. Auton. Nerv. Syst., 50, 181-188 (1994).

Physiological Transport Responses to High Intensity Exercise and Hydrostatic Pressure Gradients in Humans

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Funding:

Project Identification: 199-14-17-05 Solicitation:

Initial Funding Date: 4/94 Expiration: 4/97

FY 1995 Funding: $92,000 Students Funded Under Research: 2

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; and further clarify the role of exercise mode in this fluid redistribution by using both concentric and eccentric cycle ergometer exercise. 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. 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 constant 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. 4) Examine the effect of high intensity exercise on the capacity for fluid transfer from extravascular spaces to the circulation during orthostatic stress. In these experiments, we will examine the hypothesis that high intensity

exercise enhances the rate of fluid transfer from the tissue to the blood during acute hypovolemia induced by lower body negative pressure.

Over the past year we have addressed two important aims of our NASA grant related to mechanisms of plasma volume expansion following intense exercise. First, we have characterized the movement of albumin and fluid, the Starling factors, which contribute to this fluid movement into the vascular compartment immediately following intense exercise. Second, we have examined how varying the hydrostatic pressure gradients influences the movement of albumin and fluid following intense exercise. Finally, we have initiated experiments designed to measure changes in transcapillary exchange rates of albumin and water following intense exercise. The first two accomplishments are in there final stages of data analysis. In general our findings point to a strong role of hydrostatic pressure gradients in the immediate and preferential shift of fluid into the vascular compartment following intense exercise while colloid osmotic pressure gradients required more time to develop. Our early results had suggested such a trend and influenced our progress on the grant by having us implement measurements of capillary filtration coefficient to assess bulk transport mechanisms in conjunction with estimates of albumin transcapillary exchange. In addition, we felt it necessary to measure capillary pressure to understand the contribution of hydrostatic pressure gradients in this response. We have implemented a simple non-invasive method to estimate capillary blood pressure using rapid venous occlusion to complement our other measurements. At present we have limited data which supports the idea that the transcapillary escape rate of albumin is reduced following intense exercise. This reduction in albumin loss from the vascular compartment will contribute to preferential water retention in the vascular compartment.

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.

Molecular Mechanisms Regulating IGF-I Synthesis in Bone

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No Co-I's Assigned to this Task

Funding:

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FY 1995 Funding: $150,549
Students Funded Under Research: 1

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: 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), 2) Characterize nuclear protein factors from Ob cells that interact with basal and PGE2 response elements within the IGF-I promoter, and 3) Determine if a cause and effect relationship exists between mechanical force, PGE2 (and cAMP) induction, and IGF-I promoter utilization.

Specific aim #1 has been accomplished, and the results were published in the September issue of Endocrinology. Briefly, we have localized a potential 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. Our evidence derives from three principal observations: (1) a transfection construct containing only 122 nucleotides (nt) of promoter 1 and 328 nt of the 5' UTR retained full PGE2-stimulated reporter expression; (2) maximal PGE2 driven reporter expression required the presence of nt +196 to +328 of exon 1 when tested within the context of IGF-I promoter 1; (3) co transfection of IGF-I promoter-luciferase-reporter constructs with a plasmid encoding the catalytic subunit of murine cAMP-dependent protein kinase (PKA) produced results comparable to

those seen with PGE2 treatment, while co-transfection with a plasmid encoding a mutant regulatory subunit of PKA that cannot bind cAMP, blocked PGE2-induced reporter expression. DNase I footprinting of the 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 now have extended this observation, and have 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. 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 new data have been submitted as an abstract to the 10th International Congress of Endocrinology. Relevant to these studies is our analysis of promoter elements involved in PGE2 -stimulated IGFBP-5 synthesis. Interestingly, an unrelated (AP-2 binding motif) appears important in protein kinase A stimulated IGFBP-5 promoter activity. However, PGE2 also enhanced IGFB-P-5 stability two-fold. The resulting transcriptional and post-transcriptional effects of PGE2 works together to stimulate IGFBP-5 gene expression.

Research related to Specific aim #2 is currently in progress. We now can reproducibly prepare nuclear protein extracts for analysis of proteins that bind to this novel CRE. We are trying UV cross-linking of 32P-labeled oligonucleotide from the shifted band prepared in the gel mobility shift assay to examine the relative molecular mass of the PGE2 induced shifted band. Once cross-linked, a denaturing polyacrylamide gel determination of the apparent molecular weight of our unknown DNA binding protein will be carried out. Western immunoblotting will aid in further identification.

Specific aim #3 involves testing the effect of mechanical strain on the expression of IGF-I promoter activity. We have been delayed in initiating this area of research because we have been waiting for the delivery of the Flexercell® FX-3000. The unit has been ordered and shipping has been promised within the next month. When the unit arrives these studies will begin immediately.

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 bed rest 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 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.

Publications, Presentations, and Other Accomplishments:

McCarthy, T.L., M.J. Thomas, M.Centrella, P. Rotwein "Regulation of insulin-like growth factor I transcription by cyclic 3', 5'-monophospate (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).

Effect of Gravity on the Regulation of Circadian Rhythms

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Funding:

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FY 1995 Funding: $132,765 Students Funded Under Research: 5

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 affects 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 2G 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 2G does act as a synchronizer for circadian rhythms, it will also entrain the expression of protein synthesis within the SCN.

During the first year of the NASA Grant, several of the specific aims have been accomplished. Our previous studies have demonstrated that continuous exposure to 2G via centrifugation abolishes the circadian rhythms of heart rate, body temperature, and activity in rats for approximately 2-3 weeks. However, it is not known whether the loss of circadian rhythms following 2G exposure is due to an effect on the neural pacemaker (i.e. SCN). Therefore, we have examined whether a 2G pulse (1-3 hours) will phase shift circadian rhythms. We have demonstrated that rats exhibit a significant phase shift following 2G exposure, suggesting that the neural pacemaker has been directly affected.

We have examined the effect of a one hour 2G pulse on c-Fos expression within the SCN in order to examine the neural changes in the circadian pacemaker in response to 2G exposure. There was a significant decrease in the number of neurons that exhibit c-Fos immunoreactivity in rats exposed to 2G relative to that of controls. These results demonstrate that 2G exposure has an immediate effect on the neural pacemaker that regulates circadian rhythms, and this effect may mediate loss of circadian rhythms in rats exposed to 2G. We have also examined the effect of a 48 hour exposure to 2G on c-Fos expression within rat SCN neurons. There was a significant decrease in c-Fos expression within SCN neurons following 2G exposure relative to that of controls. These results demonstrate that the effect of 2G exposure on the neural pacemaker is prolonged, and correlated with the absence of circadian rhythms during this time. In addition, we have examined the effect of a one hour light pulse on c-Fos

expression in SCN neurons during the 48 hour 2G exposure. Control 1G rats exhibited the normal increase in c-Fos expression following a one hour phase shifting light pulse. However, when we exposed rats to a one hour phase shifting light pulse during the last hour of a 48 hour period of 2G, there was no increase in c-Fos expression in SCN neurons. These results further demonstrate that 2G exposure has a highly significant effect on the function of the neural pacemaker.

The results from the research to date compliment the planned experiments for FY96. We will examine c-Fos expression in the SCN of rats that have been exposed to continuous 2G via centrifugation for a period of 3 weeks when circadian rhythms have been shown to recover. It is anticipated that a recovery in c-Fos expression in the SCN will coincide with the recovery of circadian rhythms. We will also examine whether there will be a recovery in the effect of a phase shifting light pulse to induce c-Fos reactivity in SCN neurons after 3 weeks of 2G.

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.

Publications, Presentations, and Other Accomplishments:

Hoban-Higgins, T. M., D. M. Murakami, T. Tandon, and C. A. "Fuller Acute exposure to 2G phase shifts the rat circadian timing system." J. Grav. Physiol., vol 2, 58-59 (1995).

Murakami, D. M. and C. A. Fuller "The effect of 2G exposure on retino-hypothalamic function." Am. Soc. for Gravitational and Space Biology, Bulletin 9, 42 (1995).

Murakami, D. M., T. M. Hoban-Higgins, and C. A. Fuller "The effect of hypergravity on the circadian timing system." Biol. Rhythm Res., vol 26, no 4, 425 (1995).

Distributed Decision Making in Extended Space Flight

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Funding:

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FY 1995 Funding: $342,391

Students Funded Under Research: 0

Joint Participation: FAA, DoD

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, related 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: communication medium, 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 outcomes, cooperation, and negotiation. Products of this effort will include recommendations for procedures, systems and training principles that enhance the quality of team decision making.

Previously we developed a process model to characterize how teams make decisions in dynamic complex environments. Central to that model was a taxonomy of decisions defined by the dimensions of cue ambiguity, response availability, time pressure, and risk. Our most recent work sought to validate the model and to determine the influence of experience and crew role on professional pilots' use of situational features in decision making. Three studies involving a sorting task were conducted. Pilots were found to pay attention to aspects of the situation consistent with their crew roles: Captains focused on risk and time pressure; first and second officers focused on response determination and time pressure. Crew role rather than experience level determined feature salience. The model also guided analysis of pilots' performance in a dynamically evolving decision task. Highly experienced pilots were more sensitive to the time available for making a decision than less experienced pilots. This does not mean they were always faster, but that they adjusted their decision speed to the circumstances, maintaining a more constant safety buffer. Second, more experienced pilots were more sensitive to

cues that signaled a potential problem, which led to their obtaining problem-relevant information early in the decision process. These findings validated our decision taxonomy, but suggested that the decision process model needed to be modified to reflect the importance of time pressure and risk in the situation assessment component. New experiments are planned to examine the effects of risk, time pressure and situation ambiguity on team decision processes.

Future space missions will include crews from many cultures. Despite high levels of training, deeply ingrained norms for interacting with peers, superiors, and subordinates may lead to social conflicts and cross-cultural misunderstandings. While studies have identified effective communication strategies among U.S. crews, the generality of these strategies for non-U.S. crews is unknown. Previous efforts by other investigators have characterized cultures by dimensions that affect communication, mainly power-distance and group orientation. Our research will determine culturally appropriate communication strategies to call the crew member's attention to the error. Features of the problem are manipulated: risk, nature of the error, social implication of the error, and status of the erring and correcting pilots. Several airlines with culturally homogeneous pilot populations have agreed to participate. Next, we will extend the study to culturally mixed crews.

This study addresses the cognitive demands of distributed medical decision making as it pertains to the treatment of acute trauma patients. Information requirements for remote diagnosis by patient care providers differing in level and type of expertise (anesthesiologists, surgeons, and trauma nurses) will be determined. A library of videotapes of actual shock-trauma cases has been assembled; causes have been selected that differ in type of presenting problem, ambiguity of the diagnosis, off-camera events and error recovery. Variables manipulated in the initial study include the amount and type of information presented to the decision maker: real-time vital sign overlay and level of case history description. Initial data collection shows that certain types of judgments about the patient's condition are difficult to assess (e.g., extent of injury) and that many important cues are missed due to the video medium. Diagnostic strategies include use of correlated information to compensate for lack of complete data and relying on secondary cues reflected in team activities. Results to date indicate the importance of rich case history descriptions and the difficulty of maintaining a dynamic model of the patient, possibly due to being "out of the control loop."

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 pertains to individuals and teams that operate in dynamic high risk environments. Primary Earth applications would be expected in the aviation domain, including pilots, air traffic controllers, dispatchers, and maintenance specialists who may have to pool their resources to solve problems. To date, several airlines have adopted the dynamic decision process model developed under this grant as a framework for training their flight crews to assure greater safety of the aviation system.

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 and structure of inquiry are most useful for remote diagnosis, tailored to the level of knowledge and expertise of the practitioner.

Findings are also expected to apply to other industries where technical specialists and managers must make decisions and cope with problems in high-risk dynamic environments such as management of off-shore oil platforms and nuclear power operations.

Publications, Presentations, and Other Accomplishments:

Cohen, M.S. "Naturalistic training of metacognitive skills." 38th Annual Meeting of the Human Factors and Ergonomics Society, Nashville, TN, October, 1994.

Cohen, M.S. "Can we train flight crew decision making?" Panel at the Eighth International Symposium on Aviation Psychology, Columbus, OH, April, 1995.

Cohen, M.S., Freeman, J.T., and Thompson, B. "Training metacognitive skills for decision making." Proceedings of the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 24-27, 1995.

Fischer, U., and Orasanu, J. "The influence of experience on pilots' perceptions of problem situations." Poster presented at the annual meeting of the Judgment and Decision Making Society, St. Louis, MO, November 12, 1995.

Fischer, U., Orasanu, J., and Wich, M. "Expert pilots' perception of problem situations." Proceedings of the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 24-27, 1995.

McCoy, E., Smith, P.J., Orasanu, J., Billings, C., Van Horn, A., Denning, R., Rodvold, M., and Gee, T. "Airline dispatch and ATCSCC: A cooperative problem-solving success story with a future." Proceedings of the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 24-27, 1995.

Orasanu, J. "Situation awareness: Its role in flight crew decision making." Proceedings of the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 24-27, 1995.

Orasanu, J. "Situation awareness: its role in flight crew decision making." Eighth International Symposium on Aviation Psychology, Columbus, OH, April 27, 1995.

Orasanu, J. "Training for decision making in abnormal and emergency events." Invited address at the International Civil Aviation Organization Regional Safety Seminar, Addis Ababa, Ethiopia, October 20, 1995.

Orasanu, J. "Safety in the skies: improving pilot decision making." South Bay Soaring Associates, Sunnyvale, CA, February 22, 1995.

Orasanu, J. "Decision making in the airplane." Invited address and workshop at the International Air Transport Association Seminar on Human Factors in Aviation, Bahrain, March 7, 1995.

Orasanu, J. "Overview of NASA team decision making research." Industry CRM Workshop, Seattle, WA, September 17, 1995.

Orasanu, J. "Mini-course on Crew Decision Making." Advanced Accident Investigators Workshop sponsored by the National Transportation Safety Board, Alexandria, VA, February 15, 1995.

Orasanu, J., and Backer, P. "Performance under stress in military operatons. In: Performance Under Stress." Edited by: J. Driskell and E. Salas Lawrence Erlbaum Associates, in press.

Orasanu, J., and Davison, J. "Cross-cultural barriers to effective communication in aviation." Presentation in a panel on Facilitating Command and Control Among Multinational Forces at the Annual Meeting of the American Psychological Association, New York City, August 13, 1995.

Orasanu, J., and Fischer, U. "Finding decisions in natural environments: The view from thje cockpit. In: Naturalistic Decision Making." Edited by: Zsambok, C., and Klein, G. Lawrence Erlbaum Associates, Hillsdale, NJ, in press.

Orasanu, J., and Jobe, K. "Adaptive problem solving strategies in air transport flight crews." Paper presented at the annual meeting of the Psychonomic Society, St. Louis, MO, November 13, 1994.

Orasanu, J., and Strauch, B. "Temporal factors in aviation decision making." Proceedings of the Human Factors and Ergonomics Society Meeting (pages 935-939), Human Factors and Ergonomics Society, Santa Monica, CA, 1994.

Orasanu, J., and Strauch, B. "Temporal factors in aviation decision making." Paper presented at the Human Factors and Ergonomics Society meeting, Nashville, TN, October 28, 1995.

Orasanu, J., Cohen, M., Johnston, N., Price, A., Prince, C., and Stokes, A. "Can we train flight crew decision making?" Panel presented at the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 25, 1995.

Orasanu, J., Dismukes, R.K., and Fischer, U. Comments on vigilance by the crew of USAIR 1016 (DC-9), Charlotte, NC. Unpublished report., NASA Ames Research Center, (1995).

Orasanu, J., Rodvold, M., Smith, P., and McCoy, E. "Improving cooperative work of airline pilots and dispatchers." Presentation at the Eighth International Symposium on Aviation Psychology, Columbus, OH, April 26, 1995.

Smith, P., McCoy, E., Billings, C., and Orasanu, J. "Cooperative problem solving: Airline dispatchers and Central Flow Control." Eighth International Symposium on Aviation Psychology, Columbus, OH, April 26, 1995.

Xiao, Y., Mackenzie, C.F., and the LOTAS Group "Decision making in dynamic environments: Fixation errors and their causes." Proceedings of the Human Factors and Ergonomics Society 39th Annual Meeting (pages 469-473), Human Factors and Ergonomics Society, Santa Monica, CA, 1995.

Mechanisms of Sensorimotor Adaptation to Centrifugation

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Funding:

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Students Funded Under Research: 0

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 gravitoinertial 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.

During this first year we developed a static tilt-roll device and modified an existing laboratory rotator system to provide dynamic tilt-roll stimuli through eccentric yaw rotation (i.e. centrifugation) of human subjects. During the initial phase of testing, we recorded dependent tilt measures on eight subjects using the static roll-tilt device. The dynamic device will be used during the next two years to study perceptual and oculomotor responses during adaptation to a conflicting visual-gravitoinertial force environment. New hardware developments to date include a 1 m centrifuge extension arm, binocular eye camera system, subject restraint system, a visual display and a static roll-tilt positioning device. Our static roll-tilt tests included measures of ocular counterrolling, horizontal optokinetic nystagmus, and a new method we have been developing which involves directed saccades along perceived Earth

horizon and vertical axes in darkness. Although we have confirmed the reliability of ocular torsion and directed saccades as dependent tilt measures, we have not observed the reorientation of the horizontal optokinetic response axis reported previously by Gizzi et al. (1994).

A set of companion experiments has also been initiated using a longer (6 m) centrifuge arm at the Naval Aerospace Medical Research Laboratory in Pensacola, FL. During these studies, video eye measurements and tilt perception (using a luminous line) were recorded, and previous findings of differences in tilt response dynamics between forward facing and backward facing subject orientations were confirmed (Guedry, personal communication, 1996). Also, models of three dimensional vestibular responses during complex motion stimulation (Merfeld, 1995) have been transformed into Matlab Simulink format to enable simulations and model fits on data sets as desired.

Our near-term intent is to further study the effects of variable gravitoinertial force fields on tilt responses by comparing the static roll-tilt responses to those obtained at the same roll-tilt angles during centrifugation. Using the dependent tilt measures established in this first phase of testing, we will then proceed to examine sensorimotor adaptation to centrifugation using different combinations of gravitoinertial force and visual references.

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.

Publications, Presentations, and Other Accomplishments:

Merfeld D.M. "Modeling three dimensional vestibular responses during complex motion stimulation." Three-dimensional Kinematic Principles of Eye-, Head-, and Limb Movements in Health and Disease, Tübingen, Germany, August 27-30, 1995.

Perceived Self-Motion Assessed by Computer-Made Animations

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Students Funded Under Research: 3

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) off-vertical-axis rotation (OVAR) 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.

Work originally planned for the first year included: 1) construction of the animation movie library; 2) revision of the self-motion perception language training lessons (change from digitized-video-based to polygon-based animations); 3) development of "flow charts" for animation movie and stored verbal report selection; 4) pretesting and data collection for Experiment 1; 5) selection of a "real-time" image generator; and 6) learning to use real-time image generator. Accomplishments to date include the following: 1) a movie library of 68 animations for use with the TTD has been constructed. 2) A HyperCard stack to teach motion perception language using selected moves from the TTD animation library has been developed. 3) HyperCard stack flow charts for selection of either animation movies or

verbal reports have constructed. 4) Experiment 1 procedures were pretested in the Preflight Adaptation Trainer (PAT) Laboratory at Johnson Space Center in September, 1995. Data collection for Experiment 1, which was delayed due to moving the PAT Laboratory from Building 9 to Building 29, is now scheduled for the third week in January, 1996. 5) New, fast (120 MHz) Power PCs appear to be sufficiently powerful to serve as the "real-time image generator," as determined using the application "Virtus Walk-through Pro." An application analogous the one from Virtus but which permits easy communication using a joystick (analogous to flight simulator applications) will be identified.

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 the following: 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. 4) They permit illustrating separation of visual scene motion from self-motion.

In cooperation with Dr. L. Duckert of the University of Washington Department of Otolaryngology Clinic, the principal investigator has developed a library of animations to illustrate illusory experiences reported by patients. Specific animations selected by patients as most closely approximating their experiences are being correlated with electronystagmography findings and rotary chair test results. We anticipate examining perceptual reports using animations following challenges using off-vertical-axis and/or hearth-horizontal axis rotation in future studies.

Publications, Presentations, and Other Accomplishments:

D. E. Parker, L. G. Duckert, P. Feeney, D. L. Harm, A. K. Raj, C. Wall "Spatial orientation perception assessed by computer-generated animations." Abstracts for the Eighteenth Midwinter Research Meeting, Association for Research in Otolaryngology, St. Petersburg, FL, February 5, 1995.

D. E. Parker, L. G. Duckert, P. Feeney, D. L. Harm, C. Wall "(Abstract) Spatial orientation perception assessed by computer-generated animations." Life Sciences and Space Medicine Conference '95 - Book of Abstracts. Washington, DC: American Institute of Aeronautics and Astronautics, 1995, 30-31.

J. D. Prothero, H. G. Hoffman, D. E. Parker, T. A. Furness, M. J. Wells "Foreground/background manipulations affect presence." Proceeding of Human Factors and Ergonomics Society, San Diego, CA, 1995.

T. R. Carpenter-Smith, R. G. Futamura, D. E. Parker "Inertial acceleration as a measure of linear vection: An alternative to magnitude estimation." Perception & Psychophysics, vol 57, 35-42 (1995).

Facilitated Blood Pressure Control by Skin Cooling: Autonomic Mechanisms

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Funding:

Project Identification: 199-14-17-15 Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95 Expiration: 2/98

FY 1995 Funding: $110,554 Students Funded Under Research: 2

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 degree 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?

This project was funded in August of 1995. A new autonomic neurophysiology laboratory was established in the Noll Physiological Research Center at Penn State University. This laboratory is housed in an environmental chamber (340 ft^2) with temperature control from 0-50°C at any level of humidity. The laboratory currently includes the ability to measure, record, and analyze cardiovascular and autonomic variables in a beat-to-beat format. A noninvasive system to measure cardiac output by foreign gas rebreathing is under development and should be completed by June of 1996. Successful recordings of single- and multi-unit activity from peripheral sympathetic nerves were first made in the new laboratory in January of 1996. Development of the perfused suit technology required to reduce skin temperature has proceeded well, with successful pilot experiments being completed in February of 1996. Experimental scheduling will assume full-time status beginning May, 1996. Characterization of the autonomic responses to surface cooling will be complete within the first year of funding, maintaining planned task progress.

The central focus of this research is to develop a simplified strategy to counteract the detrimental effects of space flight on subsequent cardiovascular regulation in a 1g environment. Central to this "cardiovascular deconditioning" are two findings: hypovolemia and failure to vasoconstrict appropriately while standing. These features are functionally analogous to the hypovolemic, hypoadrenergic form of orthostatic hypotension observed in some patient populations. Simple skin cooling should be an effective means to distribute blood volume centrally and increase sympathetic activity. While relevant to the astronaut population, this technique also has application to patients with orthostatic hypotension; particularly renal dialysis patients who lose a significant portion of their total blood volume and body water during the dialysis procedure. In addressing the mechanisms to facilitate the

regulation of vascular resistance by the sympathetic nervous system, we hope these findings will translate to elderly people to ameliorate hypotension associated with postural transitions.

Publications, Presentations, and Other Accomplishments:

Bryant, K.H., J.A. Pawelczyk, X. Shi, and P.B. Raven "(Abstract) Assessment of heart rate changes during progressive exercise using power spectral analysis." FASEB J, vol 9, A358 (1995).

Colflesh, C.R., S.S. Blaker, J.H. Zuckerman, J.A. Pawelczyk, and B.D. Levine "Carotid sinus "irritability" rather than hypersensitivity: A new, more accurate name for an old syndrome." Abstract submitted, American College of Cardiology.

Levine, B.D., J.H. Zuckerman, and J.A. Pawelczyk "(Abstract) Cardiac mechanics after simulated microgravity." Circulation, vol 92, 1590 (1995).

Levine, B.D., J.H. Zuckerman, and J.A. Pawelczyk "(Abstract) Changes in cardiac mechanics (Frank-Starling relations) after two weeks of head-down tilt." FASEB J, Vol 9, A898 (1995).

Pawelczyk, J.A. "Issues confounding the interpretation of baroreflex responsiveness during dynamic exercise." Symposium presentation at the Annual Meeting of the American College of Sports Medicine, May, 1995.

Pawelczyk, J.A., and B.D. Levine "(Abstract) Cardiovascular responses to rapid volume infusion: the human Bainbridge reflex." Circulation, vol 92, 169 (1995).

Pawelczyk, J.A., and B.D. Levine "(Abstract) Limb vascular responsiveness to adrenergic agonists following physical deconditioning." Med. Sci. Sports Exerc., vol 27, S31 (1995).

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Pawelczyk, J.A., B.D. Levine, G.K. Prisk, B.E. Shykoff, A. Elliott, and E. Rosow "(Abstract) Accuracy and precision of flight systems for determination of cardiac output by soluble gas rebreathing." Am. Inst. Aeronautics and Astronautics J., (1995).

Pawelczyk, J.A., K.M. Harper, and B.D. Levine "(Abstract) Bed rest deconditioning reduces leg, but not arm, arterial compliance." Circulation, vol 92, 169 (1995).

Wilson, L.B., C.K. Dyke, D. Parsons, P.T. Wall, J.A. Pawelczyk, R.S. Williams, and J.H. Mitchell "Effect of muscle fiber type on the pressor response evoked by static contraction." J Appl. Physiol., vol 79, no 1744, (1995).

Wilson, L.B., P.T. Wall, J.A. Pawelczyk, and K. Matsukawa "Divergence of ventilatory responses to isometric contraction in anesthetized cats." Resp. Physiol., (in press).

Relation of Motion Sickness Susceptibility to Vestibular and Behavioral Measures of Orientation

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-16-17-04

Solicitation:

Initial Funding Date: 9/93

Expiration: 9/96

FY 1995 Funding: $90,000

Students Funded Under Research: 0

Task Description:

The overall objective of this proposal is to understand the relationship between human orientation control and motion sickness (MS) susceptibility. Three areas related to orientation control will be investigated. These three areas are 1) reflexes associated with the control of eye movements and posture, 2) the perception of body rotation and position with respect to gravity, and 3) behavioral utilization of sensory information for the control of postural equilibrium. All tests will be performed in normal human subjects.

Measurements of reflexes, motion perception, sensory utilization, and MS susceptibility will concentrate on pitch and roll motions because these are most relevant to the space motion sickness (SMS) problem. Vestibulo-ocular (VOR) and visual-vestibular reflexes will be measured using a unique two-axis rotation device developed in our laboratory over the last six years. Modifications to this device are proposed that will permit the measurement of otolith-ocular reflexes evoked by rotations about an axis that is eccentrically displaced from the subject's head position. Reflex experiments will place special emphasis on dynamic rotational stimuli that might evoke disconjugate torsional eye movements. This is highly relevant since recent work by Diamond and Markham (Avait Space Environ Med 62:201-205, 1991) showed a strong correlation between SMS and disconjugate torsional eye movements measured during parabolic flight. If one of our experimental dynamic rotation tests were able to reveal these same disconjugate eye movements, this could eventually lead to a cost effective ground-based test of SMS.

Motion perception will be quantified using a closed loop feedback technique developed by Zacharias and Young (Exp Brain Res 41:159-171, 1981). This technique requires a subject to null out motions induced by the experimenter while being exposed to various confounding sensory orientation cues. The magnitude and timing of reactions to changes in sensory environments provide a means of quantifying a subject's motion perception.

Posture control reflexes will be measured using moving platform posturography capable of independently altering somatosensory and visual orientation cues. The body sway of subjects exposed to sinusoidally oscillating visual field and/or platform motions under a variety of environmental

conditions will be measured. The driven sway amplitude will be used to characterize the way in which a subject utilizes particular sensory system orientation cues for postural control.

Motion sickness susceptibility will be measured by the time required to induce a defined level of MS symptoms, or by the level of symptoms at the end of a fixed period. Several MS tests will be given that evoke different levels of visual-vestibular and intra-vestibular (canal-otolith) conflicts related to pitch and roll plane motions.

The results of this work are relevant to NASA's interest in understanding the etiology of SMS. If any of the reflex, perceptual, or sensory utilization abilities of subjects are found to correlate with motion sickness susceptibility, this work could lead to ground-based tests that predict SMS susceptibility.

We are continuing to investigate the correlation of reflex parameters and behavioral responses with motion sickness susceptibility. Our preliminary experiments that attempted to quantify various orientation perception factors were disappointing, and we have decided not to pursue these experiments.

We have completed and published an extensive investigation which characterizes the influence of visual, vestibular, and somatosensory orientation cues on the control of posture. The results have been presented at two conferences and appeared in the March 1995 issue of *Experimental Brain Research.*

We also have developed a mathematical control model to explain the observed dynamic responses of visually induced postural sway. This model has enabled us to quantify how a subject uses various sensory cues for postural control. This control model and posture data were presented at the Sensory Interaction in Posture and Movement Control conference in September 1994, and published in the book, "Multisensory Control of Posture".

Our improved knowledge of postural control behavior has allowed us to develop posture test stimuli that provide quantitative estimates of an individual's preference for the use of visual, vestibular, and somatosensory orientation cues for balance control. There is a large variation among individuals in their preferences for using these orientation cues. We hypothesize that some of these various preferences or "strategies" for using sensory information could be correlated with a subject's motion sickness susceptibility. We are currently collecting posture control data that will be used to rate the strategies of each test subject, and these ratings will later be correlated with their motion sickness susceptibility.

The dynamic properties of the VOR are indicative of both peripheral vestibular receptor function and central nervous system processing of vestibular motion information. We have performed numerous preliminary experiments to design test paradigms that can efficiently identify VOR properties under a variety of conditions, with an emphasis on those conditions that have an otolith function influence. VOR response properties from these experiments will be correlated with motion sickness susceptibility.

We have converged on a test paradigm consisting of rotational motion stimuli that evoke vertical, torsional and horizontal eye movements with the subject oriented in different positions with respect to gravity. Our protocol includes an off-vertical axis rotation test that provides dynamic stimulation to the otolith organs following the decay of the VOR component due the semicircular canal stimulation.

In addition we have completed software modifications to our two-axis rotation device that enable us to investigate VOR "dumping". Data have been collected in five subjects. We are interested specifically in the shift of post-rotary eye movements from a horizontal to a vertical and/or a torsional direction that results when a test subject is tilted away from a vertical rotational axis while nystagmus is decaying. This experiment provides information about an individual's spatial orientation function by quantifying the extent to which gravity influences the direction of VOR eye movements. There is some controversy in the literature concerning the existence of this directional shift in humans. All of

our test subjects have shown some shift in direction, but there has been a large variation among the subjects.

We have completed and published in Acta Astronautica a study investigating the theoretical use of torsional vestibulo-ocular reflex measurements for identifying otolith asymmetries possibly related to space motion sickness susceptibility.

A study of threshold phenomenon related to VOR eye movements evoked by pitch and roll rotations has been completed. The results were presented at the Neural Control of Movement meeting in April 1994, and have been submitted to the *Journal of Vestibular Research.* as part of a conference paper. Results on a study comparing the dynamic response properties of vertical and torsional eye movements will be presented at the Association for Research in Otolaryngology in February 1996.

In conjunction with a NIH/NASA sponsored grant "Otolith control of posture" grant (P60-DC02072), we have been able to improve our video eye movement recording system with the addition of two new SVHS professional video tape recorders, and new smaller, lighter video cameras with greater resolution. We are continuing to make changes to improve analysis speed and accuracy of the system by taking advantage of new technology and of other researchers' contributions to the rapidly developing field of video oculography.

All aspects of this study have the potential of making significant contributions to our understanding of vestibular reflex properties, motion perception, sensory utilization, and their relationships to motion sickness susceptibility. While a great deal is known about vestibular reflex function for motions in the horizontal plane, much less is known about reflex function associated with pitch and roll plane motions where both the semicircular canals and otolith organs contribute to the vestibulo-ocular reflex. Since the current work is focused on vertical and torsional eye movements evoked by pitch and roll motions, this work will provide new baseline data characterizing 3D vestibulo-ocular reflex function in humans. This research could lead to the development of new clinical tests that provide a much more complete evaluation of human vestibular function in patients with balance disorders.

About half of all astronauts experience varying degrees of spatial disorientation or space motion sickness (SMS) in the first several days of space flight. Since current space shuttle flights are of relatively short duration, the disabling effects of spatial disorientation and SMS can impair crew performance during a significant portion of the total flight. A primary goal is to identify correlations between motion sickness susceptibility and various reflexive and behavioral measures of orientation function. An understanding of the relationship between these phenomena is relevant to efforts for developing predictions of SMS (i.e. ground-based predictive tests) and/or countermeasures to the SMS problem. As an additional benefit, the better understanding of canal and otolith function provided by this research may lead to the development of countermeasures for the more common Earth-based variety of motion sickness and motion sickness associated with abnormal vestibular function.

Finally, this research has contributed to the development of new and unique devices for characterizing human vestibular function. Specifically an unique two-axis rotation device has been developed that delivers various, controlled motion stimuli. This device allows the study of human vestibular function in three dimensions. In addition, we have developed a video-based system for the quantitative analysis of eye movements in three dimensions. This highly accurate, non-invasive eye movement recording system takes advantage of the rapid developments in video and image processing technology, and promises to be an important new tool with many different research and clinical applications.

Publications, Presentations, and Other Accomplishments:

Peterka, R.J. "Simple models of sensory interaction in human postural control. In: Multisensory Control of Posture." Edited by: Hlavacka, F., Mergner, T. Plenum, New York, pp 281-288, 1995.

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Peterka, R.J., and Benolken, M.S. "Role of somatosensory and vestibular cues in attenuating visually induced human postural sway." Exp. Brain Res., vol. 105, 101-110 (1995).

Peterka, R.J., and Benolken, M.S. "Transient postural responses induced by visual field motion in normal and bilateral vestibular loss subjects." Clinical Application of Vestibular Science, UCLA (abstract).

Peterka, R.J., and Benolken, M.S. "Somatosensory and vestibular influences on attenuating visually induced postural sway in humans." Neural Control of Movement, Hawaii (abstract).

Space flight Effects on Microbial Susceptibility to Antibiotics

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Funding:

Project Identification: 199-04-11-20
Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95
Expiration: 2/98

FY 1995 Funding: $158,666
Students Funded Under Research: 0

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.

Very little is known regarding the affects of microgravity upon the action of antimicrobial agents on common bacterial pathogens. This study has developed a simple procedure for conducting antibacterial susceptibility tests during a Space Shuttle mission. Specially prepared susceptibility test cards (bioMerieux Vitek, Hazelwood, MO) were designed to include 6 to 11 serial two-fold dilutions of 14 antimicrobial agents including penicillins, cephalosporins, a beta-lactamase inhibitor, vancomycin,

erythromycin, tetracycline, gentamycin, ciprofloxacin, and trimethoprim/sulfamethoxazole. Minimal inhibitory concentrations (MIC) of the drugs were determined by visual reading of color endpoints in the Vitek cards made possible by incorporation of a colorimetric growth indicator (Alamar blue, Sensititre/Alamar, Westlake, OH). This study has demonstrated reproducible susceptibility results when testing isolates of *Staphylococcus aureus*, Group A *Streptococcus*, *Enterococcus faecalis*, *Escherichia coli* (beta-lactamase positive and negative strains), *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*.

Plans for current year include broaden study of microbes to include several other important potential human pathogens, such as *Candida albicans*. Candidate test microbes will be evaluated to ensure that preinnoculation into Vitek cards and holding at various temperatures for 10 days will not effect their reactions to antibiotics. Additional candidate antibiotics will also be evaluated. Antibiotics affecting various sites such as cell wall, cell membrane, nucleic acids, and essential enzymes will be used to delineate effects of microgravity upon microbe-antibiotic interactions. All conditions will be optimized and the final selections of test microbes and antibiotics will be made in preparation for flight testing.

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.

Publications, Presentations, and Other Accomplishments:

Jorgensen, J.H., D.L. Pierson, S.L. Mishra, J.A. Skweres, M.L. McElmeer, L.A. Maher, R. Mulder and M.V. Lancaster "Development of an Antimicrobial Susceptibility Testing Procedure Suitable for Performance During Space Flight." 94th Meeting of the American Society for Microbiology, Las Vegas, NV, 1994.

The Effects of Exercise-Enhanced Denitrogenation on Altitude Decompression Sickness (DCS) Protection

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Funding:

Project Identification: 199-04-17-12
Solicitation: 93-OLMSA-07

Initial Funding Date: 7/95
Expiration: 6/98

FY 1995 Funding: $119,373
Students Funded Under Research: 0

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., 1993a,b: Webb et al., 1994a). This proposed 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 proposed 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 initiated. Of the 180 planned exposures 14% (26) have been accomplished as of September 30, 1995; 8% of the way to completion of the three-year program. The number of subject-exposures is insufficient to answer any questions or develop new questions. The current progress appears to be on track for timely completion, although difficulty in acquiring female subjects will slow progress at some future point. Future work will build on the subject exposures already accomplished to enable statistical analysis which will be used to answer the original question about enhancing denitrogenation with exercise.

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 the common man 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 at less cost and more quickly.

Pulmonary Deposition of Aerosols in Microgravity

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Funding:

Project Identification: 199-14-17-09
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1995 Funding: $181,508
Students Funded Under Research: 1

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 (μG) environment, sedimentation is no longer active, and thus there should be marked changes in the amount and site of the deposition of these aerosol. We propose to study the total intrapulmonary deposition of aerosol spanning the range 0.5 to 5μ in the KC-135 at both μG and at 1.8-G. This will be followed by using boli of 1.0μ 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.

Effort to date has concentrated on development of the necessary infrastructure to carry out the proposed research. Dr. Chantal Darquenne, a postdoctoral fellow from the laboratory of one of the international collaborators (Dr Manuel Paiva) joined us in July 1995. Since that time we have been determining which techniques are most suited to the performance of the proposed studies in the KC–135 aircraft. Construction of the appropriate breathing circuits has been performed, and aerosol holding containers developed. The photometer best suited to the task has been determined and is on order to arrive in February. Software to control the breathing circuit, and for data acquisition has been developed and is under test. Modifications to the design have allowed construction of a single circuit for both the total deposition studies and the bolus studies. We anticipate flying the total deposition portion of the experimental program in the second half of calendar year 1996.

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, 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.

Mechanisms of Microgravity Effect on Vascular Function

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Funding:

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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 reentry 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, reentry 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, 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.

Experiments were designed to assess the effect of microgravity on vascular contractility to 68 mM K^+ induced cellular depolarization and norepinephrine (NE). Microgravity was simulated in Sprague Dawley (SD) and Wistar (W) rats using a tail harness to elevate the hindquarters, producing hindlimb unweighting (HU). After 20 days of HU treatment, blood vessels from both HU and control rats were cut into 3 mm rings and mounted in tissue baths for the measurement of isometric contraction. HU treatment decreased the contractile response to 68 mM K^+ in abdominal aorta from W but not SD rats, compared to control. HU treatment also decreased the contraction to 68 mM K^+ in carotid arteries from both rat strains and in femoral arteries from W but not SD rats. HU treatment reduced the maximal response to norepinephrine in all arteries studied except the femoral artery from SD rats. HU treatment increased the contractile response of jugular vein from W rats to 68 mM K^+, but had no effect on that response in jugular vein from SD or in femoral vein from either rat strain. HU treatment also had no significant effect on the maximal response to norepinephrine in veins, but there was a trend toward the HU-induced enhancement of contraction. These results demonstrate that HU treatment caused a nearly

universal reduction of contractility in the arteries studied, but had either no effect or increased contractility in veins.

The next experiments to follow on this work are to assess the role, if any, of the endothelium on the effect of HU treatment. The effects of HU treatment on contractile response to cumulatively increasing concentrations of K^+ (4.9-100 mM) will also be evaluated. The present results reveal an effect of HU treatment on maximum capacity to contract but not on sensitivity to vasoconstrictor agent. Thus, future experiments will focus on possible mechanisms underlying changes in contractile capacity. The W rather than SD rat strain will be used. Future experiments will also address: 1) vascular responsiveness to neurogenic stimulation, 2) endothelium-dependent and -independent vasodilation and 3) the time course of the onset as well as the recovery from the effects of HU treatment.

Experimental Neurogenic Hypertension Program: Supplement

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Funding:

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Joint Participation: NIH/National Heart Lung and Blood Institute

Task Description:

Little is known about the neural mechanisms through which microgravity or reentry into a terrestrial environment affect regional cerebral blood flow (rCBF), even though a major challenge to reentry is the cerebral hypoperfusion associated with orthostatic hypotension. We investigate here mechanisms by which the brain may act to rapidly adjust to hypoxia to increase its blood flow. This study tests the hypothesis that the immediate vasodilation elicited in the cerebral cortex of anesthetized paralyzed rats by systemic hypoxia and/or brainstem ischemia is in part neurogenic and consists of two elements. One, reflexive, results from hypoxia excitation of neurons of the rostral ventrolateral medulla (RVL) which transynaptically elevates cortical rCBF, probably by exciting a small population of cortical neurons in Lamina V ("vasodilator neurons") whose activity initiates vasodilation. The second is a direct effect mediated by hypoxic stimulation of specific cortical neurons, possibly the same ones excited reflexively from RVL.

Study I investigates the reflexive hypoxic vasodilation, seeking to determine whether: (a) local microinjection of sodium cyanide (NaCN) into RVL will elicit a transient, reversible, site-specific, and dose-dependent increase in (rCBF); (b) whether the elevations in rCBF are associated with an increase in the frequency of burst-cerebrovascular wave complexes; (c) excitation of vasodilator neurons of Lamina V; and (d) whether lesions of RVL will attenuate the increases in rCBF and associated neuronal excitation elicited by hypoxia.

Study II investigates whether a population of cortical neurons, like oxygen sensitive neurons of RVL, may also be directly excited by hypoxia and/or NaCN, and that such activity elicits cerebrovascular vasodilation. Study II therefore seeks to determine whether: (a) the local application of NaCN to the cerebral cortex can elicit a transient, reversible, reproducible, stable, and dose-dependent increase in local rCBF not replicated by H^+, lactic acid, and not due to release of excitatory amino acids and which is of neural and not vascular origin; (b) NaCN applied subdurally and/or iontophoretically excites a subpopulation of cortical neurons which are also excited by systemic hypoxia after inactivation of RVL; and (c) the vasodilation elicited by the response to local NaCN and hypoxia is abolished site-

selectively and reversibly by local application of tetrodotoxin, while retaining cerebrovascular autoregulation and responsivity to hypercapnia.

During 1995 the principal question of Study I was answered: Does hypoxic excitation of neurons of rostral ventrolateral medulla (RVL) increase rCBF? In rats anesthetized with isofluorane, instrumented for recording of arterial pressure (AP), and artificially ventilated with the mixture of O_2, N_2 and CO_2, we established that microinjection into RVL of NaCN (300 pM) in normoxic (PaO_2=102±12 mmHg) conditions increased cortical rCBF within two sec to a maximum of 61±22% at 32±15 sec and decreased cerebrovascular resistance (CVR) by 16±7%. In parallel AP reached a peak of 31±16% in 7±1 sec. rCBF and AP gradually returned to the basal level in 170±115 sec. After spinal cord transection (10 animals), in response to NaCN microinjection, rCBF increased by 27±4% in 14±3 sec and restored within 146+48 sec. Multiple microinjections of NaCN demonstrated that increase in rCBF can be evoked from the limited area of RVL. The response of rCBF to cyanide microinjected into RVL was dose-dependent (150-600 pM) and reproducible. Microinjections of dinitrophenol (1-10 nM) (5 animals) increased rCBF similarly to cyanide. Hyperoxygenation (PaO_2: 456±93 mmHg) attenuated (from 27±4% to 17±5%, $p<0.01$) the increase in rCBF evoked by cyanide. In parallel with the elevation of rCBF, in response to cyanide microinjected into RVL, the power of 6Hz ECoG band significantly ($p<0.05$) increased. Simultaneously, the cortical vasodilator neurons, which excite synchronously with cortical vasodilatory events: spontaneous cerebrovascular waves or vasodilation evoked by stimulation of RVL, increased their activity from 2±1 sp/sec to 5±2 sp/sec (n=4, $p<0.05$). These neurons were comparably excited during hypoxemia (PaO_2: 27±5 mmHg) from 2±1 sp/sec to 6±2 sp/sec (n=3, $p<0.05$). In order to determine participation of cyanide sensitive sites of RVL in rCBF response to hypoxia we bilaterally electrolytically lesioned these sites in 5 animals. The rCBF response to hypoxemia (increase by 98±9%, PaO_2: 28.2+4 mmHg) was significantly ($p<0.05$) attenuated (by 65±12%) by lesion. Microinjection of tolbutamide(10-600 pM, 5 animals) or glibenclamide (5-200 pM, 5 animals) into RVL dose-dependently reproducibly and site-specifically within 2 sec increased rCBF (maximum by 10±4%, $p<0.01$, 5 animals) and AP (maximum by 15±2%, $p<0.05$) while CVR decreased by 6±2% ($P<0.05$). After reaching maximum in 8 sec rCBF and AP, in parallel, returned to the baseline within 280±103 sec. In spinalized animals (n=5) tolbutamide (300 pM) increased rCBF by 7±1% ($p<0.05$).

This research seeks to examine how the cerebral circulation responds to stimuli which would relate to microgravity, namely possible alterations in cerebral perfusion during adjustments to the zero-gravity environment and, perhaps more relevantly, to the risk of cerebral hypoperfusion during return to a terrestrial environment. The regulation of the cerebral circulation in relation to microgravity was identified as a major research problem in the report of the Task Force relating the missions of NASA and the National Heart Lung and Blood Institute (NHLBI) to circulatory control. Thus, the theme of the proposed project directly relates to missions of NHLBI (as encompassed in our HL18974 Program) and NASA.

The project directly investigates whether much of the cerebrovascular vasodilation and elevated blood flow initiated by altered perfusion (primarily hypoxia and/or brainstem ischemia) is the result of direct stimulation of neurons which are rapidly and reversibly excited by low oxygen, and hence act as oxygen sensors, and whose activity leads to neurogenically increasing rCBF.

The research will yield a new understanding of regulation of rCBF in hypoxia and of neuronal mechanisms responsible for the cerebral vasodilation with orthostatic maladaptation. This then sets the stage to identify (in future studies beyond the scope of this project) the transmitters and the receptors which are functionally involved. Such information may lead to the development of rational drug treatments which may facilitate the neurogenic vasodilation and counteract, at least acutely, the cerebral effects of hypoperfusion. Such approaches may be of importance in overcoming some cerebrovascular consequences of adaptation to space and to reentry to a terrestrial environment.

Publications, Presentations, and Other Accomplishments:

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The Sympathetic Nervous System in the Anemia of Weightlessness

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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 ß-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 bed rest, 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 bed rest 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 bed rest.

In the course of these investigations of anemia, an unexpected but very significant observation has recently been made that will expand our future work scope. The movement of fluid from the vascular

compartment to the interstitial space during standing is a well-established phenomenon in healthy subjects. The resulting decrease in plasma volume causes hemoconcentration of various components of the blood (Hct, Hb, protein, etc.). Some 10 to 20 minutes need to elapse before an apparent dynamic equilibrium can be attained. Patients with orthostatic intolerance (OI) of unknown cause have delayed development of orthostatic symptoms after standing. Therefore, the purpose of this study was to determine the dynamic plasma volume changes upon standing in OI patients compared to healthy subjects. We assessed postural changes in plasma volume in 14 patients with OI and 11 healthy subjects before and for up to 60 min following assumption of upright posture using the Evans blue dye method for baseline absolute plasma volume, together with monitoring of serial hematocrit and total protein for subsequent relative volume changes. Healthy subjects reduced the plasma volume 12% (range: 10-18%) while patients with OI reduced their plasma volume 21% (range: 15-27%) ($p<0.003$). OI patients required 24 minutes for their orthostatic plasma volume to plateau, whereas normal subjects only required 12 minutes ($p<0.002$). These data indicate that profound dynamic changes in orthostatic hypovolemia correlate with the development of symptoms in OI and may offer new insight into the mechanisms of orthostatic symptoms in this and in other diseases.

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 bed rest. 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 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.

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Mechanisms of Antiarrhythmic Drug Action

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Funding:

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Joint Participation: NIH/National Heart Lung and Blood Institute

Task Description:

The overall goal is to determine 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 supplement 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 a2 agonist clonidine, and prolonged bed rest 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 supplement proposal is highly complementary to the aims of the extant Program Project. By developing new techniques to study sympathetic inhibition, the proposed 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.

So far we have identified the dose of guanadrel which significantly reduced plasma levels of norepinephrine (Specific Aim 1A). This was an essential requirement for the performance of the subsequent specific aims. We have determined that 15 mg t.i.d. will reduce sympathetic tone by more than 50% and, therefore, this dose is currently in use for the ongoing bed rest studies. In addition, this regimen seems to be well-tolerated by the subjects.

Currently we are performing a modified bed rest study which allows us to evaluate whether two different models of weightlessness evoke physiologic and biochemical changes similar to those observed during microgravity. The major modification in our bed rest protocol consists of the reduction of days (from 19 to 10). With this, we have been able to increase the number of subjects able to participate in the protocol while still allowing us to evaluate the changes in sympathetic tone.

We have also made significant progress in evaluating patients with autonomic dysfunction and orthostatic intolerance that serve as a model for the cardiovascular changes observed in astronauts after space travel. After several days in microgravity, return to Earth is attended by alterations in cardiovascular function. The mechanisms underlying these effects are inadequately understood. Three clinical disorders of autonomic function represent possible models of this abnormal cardiovascular function after space flight. They are (1) pure autonomic failure, (2) baroreflex failure, and (3) orthostatic intolerance. In pure autonomic failure, virtually complete loss of sympathetic and parasympathetic function occurs along with profound and immediate orthostatic hypotension. In baroreflex failure, various degrees of debuffering of blood pressure occur. In acute and complete baroreflex failure, there are usually severe hypertension and tachycardia, while with less complete and more chronic baroreflex impairment, orthostatic abnormalities may be more apparent. Orthostatic intolerance is the cause of significant disability in otherwise normal subjects. Orthostatic tachycardia is usually the dominant hemodynamic abnormality, but symptoms may include dizziness, visual changes, discomfort in the head or neck, poor concentration, fatigue, palpitations, tremulousness, anxiety and, in some cases, syncope. It is the most common disorder of blood pressure regulation after essential hypertension. There is a predilection for younger rather than older adults and for women more than men. Its cause is unknown; partial sympathetic denervation or hypovolemia have been proposed.

We tested the hypothesis that reduced plasma renin activity, perhaps due to defects in sympathetic innervation of the kidney could underlie a hypovolemia giving rise to these clinical symptoms. Sixteen patients (14 F, 2 M) ranging in age from 16 to 44 years were studied. Patients were enrolled in the study if they had orthostatic intolerance, together with a raised upright plasma norepinephrine ($\geq$600 pg/ml). Patients underwent a battery of autonomic tests and biochemical determinations. There was a strong positive correlation between the blood volume and plasma renin activity ($r=0.84$, $p=0.001$). The tachycardic response to upright posture correlated with the severity of the hypovolemia. There was also a correlation between the plasma renin activity measured in these subjects and their concomitant plasma aldosterone level.

Hypovolemia occurs commonly in orthostatic intolerance. It is accompanied by an inappropriately low level of plasma renin activity. The degree of abnormality of blood volume correlates closely with the degree of abnormality in plasma renin activity. Taken together, these observations suggest that reduced plasma renin activity may be an important pathophysiologic component of the syndrome of orthostatic intolerance. Overall, careful autonomic studies of human subjects with autonomic disorders will permit us to better understand and treat the pathophysiologic changes brought on by microgravity environment.

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 new environment. Developing Earth-based models for changes produced by space travel will allow us to be better prepared to design countermeasures. In addition to a better understanding of disease processes, the design of new guidelines for the rational use of some medications.

Publications, Presentations, and Other Accomplishments:

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Vestibular Contributions to Post-Spaceflight Orthostatic Intolerance: A Parabolic Flight Model

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Task Description:

It has recently been observed that 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) crew mates, even when fluid losses attributable to nausea or emesis appear to be 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 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, parabolic flight on NASA's KC-135 aircraft is being used as a stimulus to generate quantifiable motion sickness in susceptible test subjects. Changes in responses to various provocative autonomic cardiovascular stimuli (including Valsava maneuvers, carotid barocuff studies, heart rate variability tests, and head-up tilt) after the onset of motion sickness in motion sick-susceptible subjects are then being compared to the changes that occur, if any, in nonmotion sick-susceptible subjects who experience the same parabolic flight pattern.

The two principal objectives of this study are: 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 are: 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.

As of February 1996, nine of twenty unmedicated test subjects have completed our 40-parabola KC-135 protocol. Of these nine subjects, three (two men, one woman) have been classified as motion sickness-susceptible, and six (five men, one woman) as motion sickness-resistant. The three subjects classified as "susceptible" each experienced at least one bout of frank vomiting while flying the parabolas. Two of these subjects were "very susceptible" (i.e., severe vomiting throughout most of the flight, with persistence of symptoms into the postflight testing period), and one was "moderately susceptible" (i.e, 1-2 bouts of emesis, toward the end of the flight only, with rapid resolution of symptoms). All three susceptible subjects were "rookie flyers". Of the six resistant subjects, all had minor or transient symptoms (i.e., salivary retention, mild epigastric awareness, headache or mild nausea), but none vomited. Two of these non-susceptible subjects were "rookie flyers", and four were "veteran flyers".

Baseline salivary amylase levels have been measured in all nine subjects preflight. Interestingly, the two highest levels in the group were found in the two "very susceptible" subjects. This preliminary finding is similar to the findings of Gordon, et al, for subjects experiencing either a seasickness stimulus or a cross-coupled (Coriolis) stimulus.

Thus far, no clear-cut relationship between acute motion sickness severity and orthostatic intolerance (defined as an inability to tolerate 30 minutes of 80-degree head-up tilt) has been identified. Of the nine subjects tested, only one had orthostatic intolerance preflight. (a typical vasovagal reaction in the female subject "very susceptible" to motion sickness). Post-flight, two subjects had orthostatic intolerance. These subjects included the male "very susceptible" subject (still feeling motion sick at the time of the test) and one non-susceptible (not significantly motion sick) male "rookie flyer" with the third-highest basal salivary amylase level. The post-flight orthostatic intolerance experienced by these latter two subjects is best described as "nonvasovagal". For example, both had a tendency toward higher sustained heart rates, higher arterial pressures, and higher cardiac outputs during the post-flight upright tilt test (compared to the preflight upright tilt test) prior to adamant requests for premature termination during the post-flight test. Post-flight upright stroke volumes and peripheral vascular resistances in these two subjects (measured by impedance cardiography) did not increase versus preflight, whereas in all of the other subjects - i.e., tilt tolerant subjects - one or the other of these parameters always did increase post-flight versus preflight. One interesting objective sign exhibited by all three intolerant subjects (one preflight and two post-flight) was a severe dimunition in cerebral blood flow velocity measured by transcranial Doppler just prior to premature tilt-test termination.

Data pertinent to the effects of motion sickness on Valsalva responses, carotid-cardiac baroreflex responses, and heart rate variability are currently being analyzed. Although statistically incomplete, power spectral density analyses of the R-R intervals of three subjects (one "motion sick", two "non-motion sick") have been performed. These data were collected during controlled frequency breathing immediately prior to takeoff and immediately after landing in both the supine and seated positions. In the motion sick susceptible subject, 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) were all increased post-flight (i.e., in the motion sick condition) compared to preflight. However, the rise in high frequency power in this subject was proportionately much greater than the rise in low frequency power, suggesting parasympathetic predominance post-flight. In the two "non-motion sick" subjects, there was a decline in total power, a decline in high frequency power, an increase in low frequency power, and a decline in very low frequency power (post-flight, compared to preflight). This rise in the low frequency power, together with the fall in high frequency power, resulted in a higher "low-to-high frequency ratio" in these sickness-resistant subjects, suggestive of sympathetic predominance post-flight. Further data collection and statistical analyses will be helpful in determining whether these trends are consistent across all motion sick and non-motion sick subjects.

Inflight data, although preliminary, have also revealed some interesting trends. First, for all subjects, beat-to-beat systolic, diastolic, and mean arterial pressures (seated) tend to decline during 25-second micro-Gz periods and rise during 50-second hyper-Gz periods. The decline in arterial pressures during micro-Gz occurs within five seconds of exposure and is likely baroreflex-mediated. It is accompanied by: 1) initial decreases in heart rate which reverse to some extent toward the end of the microgravity period as arterial pressures continue to fall and inverse baroreflexes are activated; 2) large rises in end diastolic volume and stroke volume; and, 3) large falls in peripheral vascular resistance. (Conversely, the rise in arterial pressures during hyper-Gz periods is accompanied by initial rises in heart rate that are attenuated toward the end of the hyper-Gz period as pressures continue to rise, large falls in end diastolic volume and stroke volume, and large rises in peripheral vascular resistance.)

For those subjects who were physically able to perform repetitive seated Valsalva tests during the flights (seven of the nine subjects), there was a fairly consistent difference in Valsalva-related arterial pressure responses during micro-Gz when compared to those found during hyper-Gz or during 1-Gz. Specifically, when compared to responses obtained during 1-Gz or during hyper-Gz, late-phase II arterial pressure recoveries during micro-Gz were quite attenuated. The opposite was true for late phase-II arterial pressure recoveries obtained during hyper-Gz, where such responses were amplified compared to late phase II recoveries obtained during either 1-Gz or during micro-Gz. Although it is difficult to ascertain the exact etiology of these changes, we suspect that the attenuated late-phase II recovery during micro-Gz exposure is at least partially due to generally falling arterial pressures during that phase of the parabola (see discussion in the previous paragraph). This same reasoning, applied conversely, might help explain the enhanced late-phase II recoveries obtained during hyper-Gz , since arterial pressures/peripheral resistances are generally rising during that parabolic phase. These somewhat simplistic explanations, however, are complicated by the fact that phase IV arterial pressure overshoots do not appear to be significantly greater during hyper-Gz (compared to micro-Gz or 1Gz), and early phase II arterial pressure falls do not appear to be particularly enhanced during micro-Gz (compared to hyper-Gz or 1-Gz). Further data collection and analyses will help to clarify these issues.

Earth benefits of this research include an enhancement of our understanding of the role that the inner ear (vestibular apparatus) plays in regulating the human cardiovascular system, particularly as it relates to orthostatic tolerance. Information gained from this research may prove useful in the development of new therapeutics for two common medical problems: motion sickness and orthostatic intolerance.

Integration of Multidisciplinary Sensory Data

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Joint Participation: NIH and Human Brain Project

Task Description:

The main aim of this grant is to develop a comprehensive model of the olfactory pathway as a paradigm for basic steps in sensory processing. The model will be based on a shared database consisting of multidisciplinary experimental data from molecular biology, neuroanatomy, electrophysiology, and pharmacology. The goal will be to use this data to construct compartmental models of the fundamental steps involved in processing in the sensory pathway, including sensory transduction, the formation of topographical maps, functions of modular units, and the operations of synaptic microcircuits, and to use these basic steps for a network model of the entire system. Its simple structure makes the olfactory pathway an attractive subject for this goal. Recent experimental breakthroughs by a number of laboratories including the group associated with the PI make this integrative approach to the system possible, but progress is blocked by the lack of enabling technologies for effectively interconnecting this diverse group of laboratories and enhancing their ability to format exchange data. These enabling technologies are needed at three levels: 1) data exchange between different laboratories currently working on similar problems; 2) formatting and integrating data from different disciplines into a shared database, and 3) new formats for the shared database and associated integrative tools that will facilitate more efficient data exchange and model construction. The enabling technologies involve three networked groups: the core laboratory of the PI; local area nets that tie the PI's laboratories to several Yale laboratories; and distant electronic collaborations with distant collaborators. All three networking levels will provide for integration of multidisciplinary experimental data into compartmental and circuit models. A major innovative aspect of this project is the close collaboration of a recognized center for informatics research with this group of experimental neuroscientists. This should lead to the development of new and more efficient types of databases for electronic exchange and for direct inputting into models, and the ability to use the models to carry out immediate and on-line tests of critical hypotheses. These improvements in the

application of enabling technologies to experimental data should have wide application to other areas of neuroscience. The results will provide models that will serve as critical tests of current theories of sensory processing.

Our initial focus has been on developing: 1) a database for olfactory receptor molecules; 2) tools for constructing compartmental models of dendritic functions, including dendritic spines; 3) tools for constructing and exploring compartmental models over the internet. Our current initiatives are: 1) parallel databases for molecular models of the olfactory receptor molecules; 2) construction of a database for the molecular properties of different types of neurons; 3) construction of a parallel database for canonical models of local circuits for different regions of the nervous system.

We have continued to use the olfactory pathway as a simple system for construction of multidisciplinary databases (receptor protein sequences, stained neurons, electrophysiological recordings). We are developing tools for 1) efficient analysis of the data; 2) exchange of data between laboratories, and 3) construction of computational models of neurons and synaptic circuits underlying information processing in the nervous system.

The three components (neuroscience, informatics, neuroinformatics coordination) of the SenseLab Project are in place. This is reflected in the Steering Committee: G. Shepherd, responsible for oversight of the neuroscience data and neuronal modeling; P. Miller, oversight of database and informatics technology; and B. Peterson, coordination between the neuroscience and informatics groups in developing tools for database construction, data analysis and model construction.

Supported by this grant, the Working Group has established network connectivity and network-based resources for our laboratories at Yale as well as for connecting to laboratories outside Yale. High-speed ethernet links allow effective use of 1) Sybase and Illustra central database servers; 2) Appleshare storage on large Sun disks, allowing efficient storage, sharing and daily backup of gigabytes of data and other resources; 3) A Web server, with publicly available information about SenseLab as well as limited private data for internal use only; this also appears as a component of the central Human Brain Project registry on the Scripps Web server; and 4) An anonymous ftp server, as an alternative mechanism for the exchange of information and resources.

The information that is processed by olfactory circuits is contained in odor molecules. Computer-based analysis of odor molecule-odor receptor interactions using molecular modeling and correlated mutation analysis is providing working hypotheses for critical residues in the receptors which transduce the information carried in odor molecule determinants. Many laboratories are generating the sequence data for these and other types of analysis, but there is no coordination of these efforts.

We have therefore built a network-accessible Olfactory Receptor Database, using Sybase System 10, a powerful relational database server based on Structured Query Language (SQL), resident on a workstation platform. The database is network-accessible on the world-wide web (WWW) via CGI scripts written using Sybperl. These scripts serve as a bridge between Sybase and WWW.

The database is now available in public and restricted parts. Published sequences can be accessed by anonymous users. Unpublished sequences can be accessed by laboratories who have agreed to share their data. The latter database should help to prevent duplication of research by different laboratories on the same clone. The database provides a resource to facilitate sequence comparison, phylogenetic analysis, and homology screens. It serves as a model for a broader database of the superfamily of G-protein receptors, which represent probably the largest gene family and have wide biological and clinical importance. In addition to its parent SenseLab server, the Olfactory Receptor Database will include links to the Human Brain Project (through the Scripps server) and the Association for Chemoreception Sciences (AChemS).

A long-term goal of the project is establish an Olfactory Model Database, which will integrate the data from many disciplines relative to each type of neuron and circuit. We are starting to build an initial prototype of this database using ILLUSTRA (Illustra Information Technologies, Inc., Oakland, CA), a hybrid database which combines relational database (RDB) and object oriented database (OODB) capabilities. We are well aware of the relative advantages of RDBs and OODBs. We feel that the hybrid approach gives the most flexibility, combining the ability to represent complex data of RDBs with the ease of ad hoc query and schema restructuring of OODBs. The ILLUSTRA dialect of SQL is extensible in the sense that new data bytes and new operators can be added, and operators can be flexibly redefined for any data type. We are preparing to build this database by recoding previous models of mitral-granule cell interactions and spine logic gates in GENESIS and/or NEURON, and building a complete model of the olfactory receptor neuron .

The laboratories of C. Greer and M. Ross have continued to explore effective means for exchanging data and software programs for reconstructions of synaptic terminals. We have continued to develop tools for constructing more efficient databases of electrophysiological data from olfactory receptor neurons, olfactory bulb slice preparations, and human cortical slice preparations, for more ready accessing of data to be incorporated into the computational models. A set of tools developed by B. Peterson for analysis of barrel cortex is being adapted for application to olfactory glomeruli.

Our first focus has been on a complete quantitative model of the olfactory receptor neuron. Using GENESIS, we have developed two tools which aid the user in defining conductance channels. One is a HyperCard stack with pull down menu of parameters; a click on the button generates a GENESIS file which allows these parameters to be incorporated into the compartments of the model. The other written in C supports more equation forms, giving graphic feedback showing the effects of parameter changes. We are also collaborating with M. Hines on porting NEURON to the Macintosh.

An advantage of the olfactory system is that the input is processed directly at the cortical level. We have previously shown that active properties in distal dendrites and /or spines of cortical pyramidal cells support simple logic operations. We have defined this as a canonical model, and are using it to test the hypothesis of Softky and Koch that distal dendrites function as coincidence detectors. Several tools have been created for analyzing the test runs and automating the extraction of these results from GENESIS models that produce the raw data.

The main significance of the work to date is that our laboratories at Yale are increasingly able to function in an electronically interconnected manner. We are creating an Olfactory Receptor Database that serves not only locally but also laboratories elsewhere engaged in common work on olfactory receptor molecules and second messenger pathways. We are thus moving toward our goal of a pilot project for how an Internet-based database can serve the needs of a small well focused research community. This is one of the steps necessary in bridging the gap between a database for a single laboratory and a national data repository for the neuroscience community. We are also developing databases from electrophysiological analysis of different types of neurons: olfactory receptors, olfactory bulb, and from human cortical slices. In addition, we are beginning to create tools for computer modeling of neurons and synaptic circuits as a first step toward identifying canonical forms of neurons and circuits that will generalize across different systems.

The plan is to: 1) continue to develop the Olfactory Receptor Database for Internet accessing of public and restricted sequences and develop complementary databases of receptor molecular models; 2) continue to develop tools for computer modeling of different neuron types, incorporating molecular data into molecular data into canonical representations of different neuron types; 3) start to develop tools for representations of canonical circuits in different brain regions, interacting with the Arbib group and others in the HBP Project; 4) adapt database and tools from analysis of barrel cortex to the olfactory glomeruli, and develop the concept of virtual experiments applied to databases for barrel cortex and olfactory neurons; and 5) interact with other H BP laboratories working in other systems, as a first

step toward developing common tools for a National Neuron and Circuit Model Database. Include data from physiological studies by ourselves and others of human cortical neurons.

The main overall benefit of this project is to obtain a deeper understanding of the basic neural mechanisms underlying human behavior and cognition. The specific benefit of this project is that it will enhance our ability to integrate the overwhelming amount of new information that is being obtained in the field of neuroscience using a wide range of different methods. This will help to identify principles in the operational design of specific regions of the nervous system, and thereby aid in the design of devices that simulate the operations of those systems. Our focus at the level of synaptic microcircuits will especially aid in the design of miniaturized devices. The most immediate benefit of our research may be in the development of chemosensory devices together with sophisticated neural circuits that can discriminate between different volatile chemicals. The recently convened NASA Symposium on "From Neurons to Nanotechnology," which the PI co-chaired, was focused on some of the new applications to miniaturized devices that are arising out of this and related research. It is an example of the practical directions in which this research is leading.

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Effects of Bedrest on Forearm Muscle Reflexes

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Task Description:

The overall objectives of this project are to examine the effects of two weeks of bed rest 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 bed rest.

The grant was initially funded in March of 1995. Cindy Hogeman (a technician and a part-time nursing student) was hired in September, 1995. We then began recruiting both subjects and patient monitors (to ensure compliance). We put our first group of three subjects in the two week head down protocol in November, 1995. To date we have completed and written the manuscript for a group of experiments examining the effects of forearm training on sympathetic nerve responses to rhythmic handgrip. Rhythmic handgrip is one of the paradigms being studied before and after bed rest, and forearm training is one of the countermeasures to be utilized to counter the effects of bed rest on sympathetic nerve responses to exercise. In this report we have observed that forearm training attenuates a number of sympathetic neural indices.

With regard to the bed rest paradigm, we have completed bed rest studies in eight subjects. To obtain this data set we received and fielded inquiries from 73 potential volunteers. We performed nerve studies on 26 subjects and were successful in 15. Six subjects withdrew after successfully gathering the pre bed rest data. One subject developed signs and symptoms of a previously contracted sexually transmitted disease on day eight of the bed rest paradigm and was accordingly removed from the study. The paradigms analyzed were static handgrip at 30% of maximal voluntary contraction (MVC) until fatigue followed by a period of post-handgrip circulatory arrest, and rhythmic handgrip at 25% MVC for ten minutes followed by two minutes of post-handgrip circulatory arrest. Analysis of this data is obviously incomplete. However, initial review of the heart rate and blood pressure responses (N=5) suggest that the heart rate and blood pressure responses during handgrip may be attenuated. This attenuation does not appear to carry over into the post-handgrip ischemic period suggesting that attenuated metaboreceptor responses are not likely to explain this finding. Based on prior literature, we doubt this finding suggests augmented baroreceptor activity. Whether these observations suggest a

decrease in central command will eventually need to be explored. Our next group of bed rest studies will be in March, 1996.

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 bed rest and the accompanying autonomic changes seen are a common accompaniment of many major disease processes. Accordingly, the study of autonomic control after bed rest has major implications for these problems.

For example, after a myocardial infarction, patients are placed at bed rest for a number of days. Our preliminary work suggests that blood pressure responses to exercise may be attenuated after bed rest. An attenuated ability to increase blood pressure will reduce muscle perfusion pressure and blood flow during exercise and thereby predispose the infarct patient to premature fatigue. It should be emphasized that it is clinical dogma that the impressive fatigue seen following a MI is due to reduced left ventricular function. Thus our observations may have important implications for a variety of non-space flight related pathophysiologic conditions.

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Microgravity: Sleep Deprivation and Autonomic Control

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Funding:

Project Identification: 199-18-17-15

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1995 Funding: $130,796

Students Funded Under Research: 2

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.

This project has just begun. Preliminary studies have been initiated, but there are no results to report at this time.

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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Funding:

Project Identification: 199-18-17-17

Solicitation: 93-OLMSA-07

Initial Funding Date: 4/95

Expiration: 4/98

FY 1995 Funding: $245,778

Students Funded Under Research: 4

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 three-year 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.

The work accomplished thus far for this project includes staff hiring, experimental set-up, subject recruitment, screening and pilot testing, and initial data collection and analyses. We have hired four part-time research assistants, and we have included in our team two post-doctoral fellows from Austria and Japan. Research assistants have been trained to conduct the various data collection and analyses tasks. We have redesigned one of our sleep reduction schedules, so that the schedules to be evaluated and compared are: polyphasic (six thirty-minute naps per day), biphasic (two 1.5 hr. sleep episodes per day), and monophasic (one 3-hr sleep episode per day). We have also optimized and made more efficient the data collection protocols in order to be able to study three subjects simultaneously as opposed to two as originally planned. Equipment has been acquired and tested, and the scheduling/performance testing protocol has been optimized. A difficult and complex task involved subject recruitment (subjects need to spend 3 months of their time in the lab over a 5 month period). Prospective subjects interviews, screening and pilot recordings have been completed successfully. We have also successfully completed the first month-long study, involving three subjects, each following a different schedule.

Data analyses are under way and have demonstrated good subject compliance and minimal loss of data due to technical or other problems. By the end of year two we anticipate studying a total of 18 subject/months.

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, and that it will form the basis for; 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.

The hypothesis formulated here are undoubtedly pioneering within the field of sleep research and work-rest management. 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 will also provide an opportunity for graduate students to be trained on the fundamental skills of sleep/performance research and related applications, who could become future professionals in sleep/work management for space and other missions.

Visual and Vestibular Contributions to Human Heading Estimation

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Project Identification: 199-16-12-37

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/96

FY 1995 Funding: $100,000

Students Funded Under Research: 3

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 examining how humans process and integrate visual motion information, how eye movements confound this problem, as well as how the vestibular system senses self-motion and interacts with the visual system. The vestibular portion of this project has made technical progress as the human sled at the Vestibular Research Facility at NASA Ames Research Center is nearly complete and ready to begin testing, although no data have yet been gathered. The visual and oculomotor portions of this project have also made considerable progress in FY95.

Dr. Thompson at the University of York in the United Kingdom and the principal investigator (PI) have identified human errors associated with low-constant motion stimuli (such as motion obscured by fog). In FY95, we tested and rejected many current models for these effects. Dr. Verghese, an NRC postdoctoral associate, and the PI have measured how effectively the information in multiple motion stimuli can be processed simultaneously and have developed quantitative models that predict the same trends found in the human performance data. In FY94, Dr. Perrone at the University of Waikato in New Zealand and the PI developed a neural model of human heading and depth estimation from the visual motion experienced during self-motion [16]. In FY95, Dr. Beutter (an NRC associate), Mr. Stassart (a Sterling Software employee), and the PI developed a rapid prototyping software package (EGOSS) for simulating self-motion stimuli which has enabled us to test this and other models. Using this package, we have shown that heading judgments during simulated motion around a curve

show systematic errors related to the sharpness of the turn and the turning rate. We are presently testing the model under identical conditions. In FY95, in collaboration with Dr. Mulligan of AFH, Dr. Beutter and the PI determined that the shape of the viewing window can cause humans to misperceive the visual motion behind the window which has considerable implications for display and cockpit design as well as our understanding of how visual cortex processes motion. In addition, by simultaneously performing classical psychophysical measurements and measuring eye movements, we have developed and validated a new analysis technique that shows that the errors in motion perception can be quantitatively predicted from the eye movements. Finally, in collaboration with Dr. Lorenceau, a visiting scientist from the University of Paris, Dr. Beutter and the PI have also shown that, under some circumstances, 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.

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 (instructors, freeway designers, etc.) interested in reducing accidents. 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.

Publications, Presentations, and Other Accomplishments:

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Countermeasure for Microgravity-Induced Muscle Atrophy

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Funding:

Project Identification: 199-18-17-10 Solicitation:

Initial Funding Date: 9/94 Expiration: 9/96

FY 1995 Funding: $190,000 Students Funded Under Research: 0

Task Description:

Prolonged exposure to microgravity results in a spectrum of physical consequences to crew members. These include cardiovascular deconditioning and skeletal and muscle atrophy. The muscle atrophy is accompanied by declines in strength and endurance far out of proportion to the decrease in muscle volume. We propose that the decrease in muscular activity against resistance results in a decline in intramuscular generation of IGF-1 which in turn results in decreased muscle structure protein synthesis.

Using horizontal bed rest as a model of microgravity-related muscular inactivity, and stable isotope labeled amino acid infusions, we have previously documented muscle atrophy and weakness are not due to accelerated muscle protein degradation. Studies performed in the past year have shown by direct methods that bed rest results in decreased muscle mixed protein synthesis. This decline in muscle protein synthesis is correlated with a-actin gene expression quantified by ribonuclease protection assays of a-actin mRNA.

In the third year of this project, we will evaluate the effect of growth hormone or epinephrine administration on muscle protein synthesis in six normal subjects and these two pharmacological countermeasures in six subjects undergoing two weeks of horizontal bed rest.

1. Direct measurement of skeletal muscle protein synthetic rate:

Our previous technique of using 13C-leucine infusion has been supplanted by the use of d6-phenylalanine as a tracer amino acid. Fourteen days of horizontal bed rest resulted in a 35% decline in fractional synthetic rate in vastus lateralis in six normal male subjects described in the appended manuscript.

2. Quantification of changes in a-actin and b-myosin mRNA in human muscle during bed rest:

There was a 30% decline in actin message coincident with a 35% decline in muscle protein synthesis in four completed subjects. Myosin message declined only 10%, and the change was not statistically significant. There was a signification positive correlation between fractional synthetic rate (determined by d6-phenylalanine incorporation) and the α-actin mRNA quantification.

The results of these studies will have impact on medical care. Our previous reports and the results of the current grant year have quantified muscular inactivity-associated atrophy and weakness and demonstrated that the mechanism is entirely due to a decrease in muscle protein synthesis. The next phase of these studies will evaluate pharmacological countermeasures which will be directly applicable to other muscle atrophy syndromes, such as trauma-related immobilization, AIDS wasting, cancer cachexia, and aging.

Publications, Presentations, and Other Accomplishments:

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Williams, W.J., Fortney, S.M., Stuart, C.A., Pietrzyk, R.A., Chen, Y.M., and Whitson, P.A. "Changes in fluid/electrolyte controlling hormones during head-down bed rest: Effect of dietary sodium." FASEB, (1994).

Adaptive Plasticity of Otolith-Ocular Responses

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Funding:

Project Identification: 199-16-12-17
Solicitation: 93-OLMSA-07

Initial Funding Date: 10/94
Expiration: 9/97

FY 1995 Funding: $193,925
Students Funded Under Research: 3

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 three-dimensional (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 linear vestibule-ocular reflexes (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. 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 was demonstrated following space flight or exposure to altered visual inputs. Following an 11 day flight, two rhesus monkeys showed 1) changes in the relationship between vergence angle and sensitivity to IA or DV motion, and 2) 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.

The nature/time-course of LVOR adaptive plasticity to motion along IA-, NO-, DV-, and intermediate axes will be characterized in proposed studies after two experimental manipulations planned for FY 96. First, visual feedback to LVOR will be changed using spectacles that: a) alter required vergence, b) change apparent interocular distance, c) magnify or minify the visual world, or d) displace vision left/right or up/down by various amounts (10-45°). Second, otolith stimuli will be altered by exposing subjects to altered gravitational fields using a centrifuge for 1 to 16 days. Understanding signal processing underlying LVORs depends on characterizing vestibular-nerve inputs to central pathways controlling VORs. These afferents differ in the spacing of their action potentials and in their response dynamics. Research described in this proposal will use a galvanic polarization paradigm to study LVORs in the presence or absence of inputs from irregularly discharging afferents. Results will provide important new insights into VOR signal processing mechanisms.

Continued analysis and preparation of LVOR adaptive plasticity data for publication. Previous studies measured eye motions that stabilize visual perception during linear head motion like that produced actively during normal behavior (posture, walking, running) or passively inside a moving vehicle. We named these responses linear LVORs. Prior to our work, it was thought that otolith (gravity receptor) eye movement control was limited to eye ball countertorsion during static head tilt. Our studies showed that there is robust, complex, visually compensatory eye position control during linear head motion over a range of stimulus frequency.

Last years' work began to characterize adaptive plasticity of LVORs during altered vision as a preliminary step to space flight and centrifugation studies. Work has been slow due to the time the Principal Investigator (PI) spent on Bion flight experiments. Preliminary studies showed LVOR adaptive changes during and after altered vision produced by displacing prisms. Preliminary results were published in abstract form, and are being prepared for publication. Results have supported our hypothesis that LVORs will be modified similarly to angular VORs modifications by visual changes. Studies in FY 96 will continue to test that hypothesis and will test whether exposure to different levels of gravity by centrifugation alters LVORs. Experiments are designed to test whether adaptation 1) is complete in all subjects, 2) has a time course, or 3) is causally related to the motion sickness which almost half of all space travelers experience shortly after entering space.

To characterize LVORs and their adaptation, it is essential to understand how the brain controls them. VORs result from 3-neuron arcs consisting of: 1) vestibular afferent cells 2) brainstem cells and 3) extraocular motor neurons. Vestibular afferents differ in discharge properties, some having regularly, and others irregularly spaced discharges. 1) Irregular afferents have more phasic-tonic response dynamics with significant phase leads at higher frequencies of head movement. 2) Irregular ones are ten times more sensitive to externally applied galvahic currents than regular ones. The role of irregular and regular afferents in modifying LVOR characteristics is being examined by inhibiting one or the other of these pathways during linear and angular stimulation using 100mA anodal(-) or cathodal(+) current through implanted 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. Use of this paradigm, developed by Dr. Minor, has shown that angular VORs are controlled by inputs from regular afferents. This might not be true for LVORs. Response characteristics of LVORs depend on gaze direction and vergence angle. Switching of irregular, more phasic, afferent inputs to central pathways controlling these reflexes would be one way to cause rapid changes in LVOR response dynamics. Pilot data supports continued testing of the afferent role in LVOR modulation. Sponsored successful application to NASA GSRP by Dr. Albert Assad, a clinical fellow in Otolaryngology at Harvard Medical School, to conduct experiments under auspices of NASA VRF. His research, "Visual-Vestibular Response to Linear Acceleration" will be conducted over the next year at MIT and the Ames Vestibular Research Facility. Coordinated planning for NIH grant application with a component for using VRF centrifuge by Dr. Dan Merfeld of the Oregon Neurological Sciences Institute.

Aging and experiencing microgravity both entail sensory and motor modifications that stimulate neuroplastic mechanisms to restore, or 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 without anatomical or physiologic compromise, although 'deconditioning' accompanies prolonged exposure to microgravity. As in the aged, such deconditioning is marked by homeostatic changes. Well-known examples in space include those related to cardiovascular and musculoskeletal systems.

Publications, Presentations, and Other Accomplishments:

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Reconstructions and Representations of Cerebral Cortex

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Funding:

Project Identification: n/a

Solicitation:

Initial Funding Date:

Expiration:

FY 1995 Funding: $

Students Funded Under Research: 0

Joint 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 cerebral cortex has made considerable progress on several fronts. One focus has been on computerized 3-D reconstructions and automated flattening of the cortical surface. We have developed a two-stage flattening algorithm that is faster and more robust than previously published methods. This method has been successfully applied to the analysis of cortical organization in both the macaque monkey and the human. Cortical flat maps also provide a natural substrate for a surface-based coordinate system, which has important advantages over conventional stereotaxic coordinates because it respects the topology of the cortical surface. We have developed separate surface based coordinates for both the monkey and the human cerebral cortex. Surface-based coordinates for human cortex provide an objective and topologically accurate framework for evaluating the vast amounts of data emerging from functional brain imaging studies. In order to deal with individual variability in cortical convolutions, we use shape-based deformation algorithms to transform one brain to match the shape of another, while respecting the topology of the cortical surface. Our current approach starts by using sulcal landmarks to drive an initial warping from one hemisphere (the source) to match the shape of another hemisphere (the target). The transformation is then refined by using a volume-based fluid deformation model to obtain a better match of the cortical grey matter throughout the reconstruction. This combined approach has been successfully applied to reconstructions of a large portion of the occipital lobe in the macaque. We have also successfully applied shape-based deformations to cortical flat maps, which is computationally less expensive than volume deformations. Finally we have developed a prototype database of connections, maps, and areas (DOCMA). This will provide a valuable framework for tracking and efficiently accessing information related to different partitioning schemes for cerebral cortex in monkeys and humans as well as information about the connections of different cortical areas.

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.

Publications, Presentations, and Other Accomplishments:

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Factors Affecting Decompression Sickness in Astronauts During Extravehicular Activity

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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 that 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 of 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 also investigate the effects of heavy exercise (weight training) on DCS risk. To date, the overall DCS incidence is 33% in 162 studies with all but three incidents being joint pain. 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 enhance nitrogen elimination and reduce the incidence of bubbles and DCS. The data also indicate that weight training and weight/height may influence DCS risk. We suggest 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 proposed studies will increase the number of trials under conditions already studied and will add new conditions to better understand the fundamental physiology of decompression and, eventually, to apply this understanding to safely reduce the time necessary to prepare for EVA.

During the period Dec. 1, 1994 - Nov. 31, 1995, we conducted eight of 24 planned altitude exposure experiments. Most of the experiments were deferred to allow repairs on a mass spectrometer needed for measuring respiratory nitrogen elimination. These repairs are now complete, and the deferred experiments will be conducted during the next 18 months.

Our plans for the coming year are to add weightlessness to the simulated EVA altitude exposures at 30,000 fsw. This will be accomplished by having the subjects in a supine position while they perform standard EVA exercises with their arms. Our hypothesis is that joint stresses induced by erect posture in a 1-g environment cause bubble formation in the legs thereby predisposing them to DCS. DCS has been almost totally confined to the legs in our experiments and in the work of others who do terrestrial

EVA simulation. In contrast, DCS predominates in the arms of immersed divers whose legs are free from gravitational stresses. Gravitational effects may help explain why DCS is common during terrestrial EVA simulation but apparently rare during EVA on orbit.

Decompression sickness can occur both on Earth and in space, and existing data suggest that humans may be less susceptible in space than on Earth. Our goals are to investigate how microgravity might affect inert gas elimination, bubble formation, and the incidence of DCS symptoms. This is accomplished by simulating microgravity during pre-EVA denitrogenation and during simulated EVA at a reduced pressure of 30,000 feet altitude. Our hypothesis is that mechanical stresses in the body in response to the forces of gravity generate gaseous micronuclei that are the focal sites for the formation of bubbles that cause DCS. These studies may help us to understand the natural process of joint wear during life in a 1-g environment because the mechanisms by which nuclei and bubbles are generated in the body appear to be the same as those responsible for lubrication.

Publications, Presentations, and Other Accomplishments:

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Adapting to Altered Gravity and Vision

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Responsible NASA Center: Ames Research Center

Task Description:

Four series of experiments are proposed, 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 adaptation to each ("dual adaptation") and an increased ability to adapt to a novel sensory rearrangement ("adaptive generalization"). In Experimental Series I, human subjects are exposed to a visual analogue to altered gravity: +/- 15- diopter prismatic displacement. Of interest in this series of experiments is the longevity of adaptive generalization (in terms of hand-eye coordination) to a 30-diopter prismatic displacement and the range of sensory situations for which adaptive generalization has an impact. In Experimental Series II, human subjects are exposed to another visual analogue to altered gravity: 108-degree rotation of the visual field. In this series, the Principal Investigator (PI) tests the efficacy of certain discriminative cues for differentiating 108-degree rotation from the normal visual environment and tests for adaptive generalization. In Experimental Series III, human subjects are exposed alternately to hypergravity (+2 Gz), in the human centrifuge, and normal gravity in an attempt to produce dual adaptation with respect to both muscle-loading (motor behavior) and the elevator illusion (visual perception). Also examined is the longevity of the presumed dual adaptation. In Experimental Series IV, adolescent Sprague-Dawley rats are exposed alternately to 7 days of continuous +2 Gz, by means of centrifugation, and 7 days of normal gravity. Measures are obtained of general activity, posture, locomotion, righting, and swimming ability. Evidence of dual adaptation and a measure of its longevity are examined. Evidence of dual adaptation, especially with respect to exposure to altered gravitational-inertial forces, and an elucidation of its controlling variables, should find application in countermeasures for the deleterious effects of microgravity on humans.

We have initiated studies of dual adaptation in four areas: 1) Altered vestibulo-ocular reflex. The aim of this investigation, which is being carried out in collaboration with Dr. Bruce Bridgeman of the University of California, Santa Cruz, is to expose human subjects to an altered relationship between

lateral head turning and visual feedback, and to measure the adaptive and dual-adaptive changes predicted to occur with respect to the vestibulo-ocular reflex (VOR). In this set of experiments, subjects actively turn the head from side to side (to the sound of a metronome beat) while viewing a spot of light in the otherwise dark laboratory. During the adaptation period, the light is caused to move in the same (or opposite) direction as the head is moving. Initially, this arrangement causes the subject's tracking eye movements to be in error due to the fact that the reflexive turn of the eyes when the head is turning in the opposite direction is unable to lock onto the target because of its instability. Previous studies have shown that as little as 5-10 minutes of such experience leads to an adaptive correction of the fixation response as well as an adaptive change in the VOR (subsequently measured by recording eye movements as the head rotates from side to side in total darkness). The experiment that we have planned entails having our subjects alternate between adapting to visual target movement in one direction and adapting to movement in the opposite direction, with the prediction that, after an indeterminate number of such alternations, subjects will exhibit accelerated adaptation and readaptation of the VOR at the point of transition between one condition and the other (i.e., dual adaptation). Unfortunately, after testing a number of pilot subjects, we have had great difficulty in obtaining VOR adaptation (let alone, dual adaptation). We believe that this problem stems from the fact that we are having subjects move the head actively, whereas almost all of the previous VOR adaptation literature is based on passive bodily rotation. To rectify this problem, we have obtained a motor-driven rotating chair, by means of which we expect to produce reliable VOR adaptation. This concern has also suggested to us the importance of making a direct comparison between actively and passively induced VOR adaptation, a study which we propose to undertake in the near future.

2) Altered visual position constancy. The aim of this investigation, which is being carried out in collaboration with Dr. Robert B. Post of the University of California, Davis, is to expose human subjects to an altered relationship between lateral head rotation and visual feedback and to measure adaptation of the perceived stability of the visual field, referred to as visual position constancy (VPC). This study is similar to the VOR study described above in that subjects rotate the head from side to side and view a visual stimulus (in an otherwise dark room) that is caused to move either with or against subject's head movement. The difference is that, instead of measuring eye position in the dark during head turning (i.e., the VOR), we are measuring apparent visual stability (VPC) of a spot of light. Thus, while at the outset of exposure to the loss of VPC the target will be appear to be in motion, continued exposure should lead to a reduction and eventually an elimination of this illusory experience. In our first experiment, we have succeeded in producing dual adaptation to altered VPC, using the frequency of the head turn as the discriminative cue. The results of this study will be presented at this year's annual meetings of ARVO. We are now in the process of collecting data on subjects whose adaptation to altered VPC is induced and measured once a week for eight weeks in a row. This procedure, we hope, will demonstrate long-term dual adaptation.

3) Altered gravitational-inertial forces. In this 8-week experiment, which was carried out last fall in collaboration with Drs. Nancy Daunton (NASA-Ames), Robert Fox (San Jose State University), and the PI, we exposed rats, by means of centrifugation, to seven days of hypergravity (2 Gz), alternating with seven days of normal gravity for a total of four such alternations (i.e., 8 weeks). Much previous research by Daunton and Fox has shown that chronic exposure to 2 Gz causes serious deficits in a number of behaviors that depend heavily on vestibular functioning. For example, the righting reflexes of recently centrifuged rats when held upside down and dropped (into a vat of body-temperature water) are abnormal (e.g., they may land on their sides), as is their subsequent swimming behavior (e.g., they may swim underwater, rather than immediately surfacing). However, these signs of adaptation to hypergravity disappear after awhile. Thus, several post-centrifugation days later the behaviors typically return to normal, indicating that complete "readaptation" has occurred. The prediction from the dual adaptation hypothesis is that the readaptation curves obtained for the second, third, and fourth transition from 2 Gz to normal gravity will be steeper (perhaps progressively steeper) than is observed on the first transition. In short, it will take less time to attain complete readaptation. Analysis of the videotapes of the subjects' righting and swimming behavior is both laborious and time-consuming and has been

ongoing for the last three weeks. In a few more weeks we should have a tentative idea of whether we were successful in producing dual adaptation for one or both of these behaviors.

4. Pitched visual environments. The following represents a new direction of our research in terms of the type of sensory rearrangement being examined, although not in terms of the conceptual framework.

It is now well known that one's visual-spatial orientation is greatly influenced by the presence of a pitched optical array. For example, when facing the interior of a box or a room that has been pitched by 20 degrees top-backward, an observer's visually perceived eye level (VPEL) is shifted upward by approximately 10 degrees (e.g., Stoper & Cohen, 1989). This is of great interest to the NASA space program because, in the weightlessness of earth-orbit or a trip to Mars, visual cues for orientation are all that the astronaut has available for spatial orientation.

We have been systematically testing the possibility that active interaction with a pitched visual environment will lead to adaptation in the form of a reduction of the effect of this environment on VPEL (and on other visual experiences). If such adaptation can be established (no one has heretofore provided the conditions likely to produce it), it will be the first step toward looking for dual adaptation in this situation. To accomplish the latter, we would have subjects first adapt to an environment pitched in one way and then to an environment pitched in the opposite direction (or placed back in the upright orientation).

We have looked now at four different conditions of active interaction within a 20-deg top-backward pitched room. We have found evidence for moderate adaptation in three of these conditions, in terms both of a reduction in the effect of the room on VPEL while the room is pitched and a negative aftereffect after the room has been returned to the vertical orientation. The effective conditions all involve having the subject learn correct eye fixation responses with respect to VPEL as he/she walks toward the back wall of the pitch room.

After assessing several more potentially effective subject-interaction conditions, we will settle on the best of the group and use this means of adaptation in an experiment aimed specifically at producing dual adaptation. If this is successful, we will then look for adaptive generalization by exposing dual-adapted subjects to a room that has been pitched in a manner that they have never before experienced.

The ability to adapt (and readapt) to altered visual and gravitational-intertial 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 from 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.

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Spatial Auditory Displays for Space Missions

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Funding:

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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 will

also be 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 first grant-year includes completion of four psychophysical studies. Two experiments investigated localization performance for virtual sources both with and without head and/or source motion, and two studies examined optimal intelligibility of spatialized speech sounds as a function of spatial position and also using the "telephone-grade" audio that is likely to be a characteristic of many real-world systems. Work was also begun on a study to determine echo thresholds using non-realtime stimuli in which the number and salience of early reflections is manipulated.

In the area of technology development, a HRTF measurement system was installed at ARC and initial measurements on individual subjects were conducted. A signal processing algorithm was also developed and demonstrated for real-time synthesis of spatial audio cues using statistically-optimized pole-zero representations of a set of HRTFs. To the extent possible, we also began the development of basic software capabilities to enable the synthesis of reflection cues in dynamic contexts. However, real time implementation of such stimuli will require the purchase of additional hardware.

Eight articles or book chapters, eight conference papers, two technical reports, eighteen technical presentations, and one patent resulted from the work of the first grant-year.

The 3-D audio research activities conducted at ARC under the grant for "Spatial Auditory Displays for Space Missions" have brought together 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 US patent recently awarded 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 7 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. 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, human-machine interfaces, simulation, communication, design and medical facilities, within contexts ranging from advanced interfaces for future space exploration to entertainment systems. For example, our industry collaborator, Crystal River Engineering, has developed products to enable the use of spatial audio technologies for virtual reality, multimedia, and video games.

Publications, Presentations, and Other Accomplishments:

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Altered Gravity Locomotion Using Differential Pressure

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Funding:

Project Identification: 199-26-12-40

Solicitation: 93-OLMSA-07

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FY 1995 Funding: $39,141

Students Funded Under Research: 1

Responsible NASA Center: Ames Research Center

Task Description:

We have developed a novel method of imposing an axial (headward or footward) external force on the body with air pressure that, by acting at the center of mass of the body, changes the "effective" body weight during standing, walking and running on a treadmill. We have previously demonstrated that this method has the capacity to generate Earth body weight in microgravity or to unload completely the body on Earth, depending on the direction of the air pressure. Because the air pressure force acts to increase or decrease body weight (at the mass center), we prefer to think of this method as simulating hypergravity and hypogravity, respectively. The purpose of this study is to investigate how gait mechanics and locomotion energetics are influenced by changing the effective body weight by this method. If our method simulates altered gravity adequately without otherwise abnormally affecting gait, then we believe this is an optimum method of modulating lower limb musculoskeletal forces. Hypothesis: We hypothesize that hypogravity and hypergravity treadmill locomotion are possible on Earth by altering the effective body weight of an individual using differential air pressure. We further hypothesize that Earth-equivalent treadmill walking and running are possible in microgravity by applying an external force with air pressure equal to one Earth body weight. Methods: The proposed work consists of three parts: (1) determination of biomechanical and physiological variables on gait speed and g-level (Study #1) ; (2) validation of Upper Body Positive Pressure as a means of loading the body during treadmill locomotion (Study #2); and (3) Simulation of 1-g locomotion during a KC-135 flight (Study #3). Study #1: Ground reaction force, EMG, , heart rate, blood pressure and kinematic data will be monitored on 5 male and 5 female subjects as they walk and run on a treadmill at 3 speeds at simulated g-levels from 0.25g to 1.5g. Study #2. Simulated hypergravity locomotion will be evaluated using UBPP. The same subjects and the identical measurements as in Study #1 will be made. Study #3. Treadmill walking and running with one-body-weight equivalent air pressure loading will be analyzed during the short "zero-g" portions of the flight. Expected results: Study #1: We expect that experimental biomechanical variables of gait to correlate well with theoretical predictions for hypo- and hypergravity locomotion. Study #2. We expect UBPP to give similar results compared to LBNP, thus providing a lightweight alternative for use in space. Study #3. We expect walking and running to be simulated well during "zero-g" phases of the KC-135 flight.

Our hypo-/hypergravity locomotion simulator is near completion. Documentation is now being prepared for submission to the Human Occupancy Review Board. Studies #1 and #2 are scheduled for the summer of 1996. Study #3, originally scheduled for Year 2, will be completed in Year 3, if a no cost extension is granted.

We believe the primary cause of bone loss in space is the reduction in level of daily mechanical loading generated by exercise in space compared to levels achieved on Earth. It is well-known that musculoskeletal forces generated by treadmill exercise in space are reduced by 60 to 70 %. The lower forces and characteristic forward-leaning running style are the result of a surface-contact restraint system that pulls the astronaut or cosmonaut to the treadmill with elastic cords or springs. With the air pressure system described in this study, we are capable in space of applying near the mass center of the body a "non-contact" resultant force equivalent or greater than one Earth-body weight. This system will allow us to test whether additional factors not related to mechanical tissue loading affect musculoskeletal adaptation in microgravity. If our hypotheses are correct, treadmill locomotion in space will be kinetically and kinematically equivalent to locomotion on Earth and musculoskeletal tissue mass and function will be conserved.

It has been shown recently that early gait therapy significantly speeds recovery and improves gait in certain patient populations. Drawbacks to current body weight support systems (BWS) are: 1) their inability to support patients comfortably when supporting a significant portion of body weight and 2) the inability to apply a constant support force centered at the mass center, enabling a more natural gait. We are exploring the use of our device in its hypogravity configuration (lower body positive pressure) as a walking assistance device during rehabilitation of gait in an effort to overcome the above problems. Initial results on healthy subjects indicate that from 0 to ~100 % of weight can be supported comfortably without adversely affecting gait. Healthy subjects tolerated lower body positive pressures well. While significant problems remain, the method shows promise of becoming a useful new technology for rehabilitation of gait following a stroke or orthopedic surgery.

Skeletal Adaptation to Physical Activity

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Funding:

Project Identification: 199-26-12-35

Solicitation: 93-OLMSA-07

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Expiration: 2/98

FY 1995 Funding: $73,703

Students Funded Under Research: 0

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 load rate as contributors to the regulation of bone density and structure. Hypothesis. 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 suggest 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). Methods. The proposed work consists of three parts: (1) correlation of densitometry data to long bone structure and strength (Study #1); (2) refinement of our GRF monitoring system and densitometry algorithm; and (3) correlation of age and physical activity to bone density and structure (Studies #2 and #3). Study #1. Ten male and ten female tibiae and radii 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 15 high loaders and 15 low loaders will be measured for two consecutive weeks using the GRF system, log books, and pedometers. Activity level defined by the different methods will be correlated to bone density and structure. Study #3. Calcaneal and tibial scans and daily GRF histories will be collected on 50 young (25 male; 25 female) and 50 elderly (25 male; 25 female) subjects. The influence of age and activity level on bone will be investigated. 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. Study #3. We expect to see the decline in activity level with age as a major contributing factor to bone loss with age in men and women. Schedule. Study #1 will be completed in the second year; Study #2 will be completed in the second year; and Study #3 will span years two and three.

First year proposal objectives included (1) determination of long bone structural and bone material elastic properties from special analysis of bone densitometry and (2) instrumentation development for quantification of physical activity by monitoring daily ground reaction forces. Densitometry: Preproposal preliminary results demonstrated highly correlated within-bone structural and material properties, but included scan interpretation errors among bones caused by entrapped air in embalmed bones and required destructive testing to obtain strain gage locations. In order to obtain very accurate and precise bone cross-sectional areal properties we: 1) refined our algorithms that filter, calibrate and process the bone density data from the high energy beam of a DXA clinical scanner, 2) designed and built a two axis digitizer with analysis software to locate gage coordinates; and 3) designed and built a water pressure flushing system to remove trapped air. Comparing preliminary surface strain data and densitometry data, mineral distribution within a cross-section accounted for all but 2% of the variation in flexural rigidity at five gaged sections along the diaphysis and metaphysis of the bone. Mean error in areal moment principal angle from densitometry versus respective flexural rigidity principal angle from surface strain measurements was 0.5 degrees. The "effective" elastic modulus, defined as the principal flexural rigidity divided by the respective principal areal moment of the bone tissue (mineral + matrix at theoretically zero porosity) was nearly constant at all sections and equal to approximately 25 GPa. These results raise new questions regarding the relative importance of micro- and ultrastructural organization of bone tissue and mineral in the determination of structural properties. For example, what is the relative contribution of cancellous bone tissue to strength and stiffness at the femoral neck? As an outgrowth of our earlier work, we have now successfully applied our line by line densitometry methods to small animal bones. This technically challenging project has extended the capability of clinical scanners and allows us to obtain complete areal moment properties of rat bones noninvasively along the entire length. Accuracy of areal moments (I_{min} and I_{max}) and principal angle registration, determined from analysis of a rat-bone-sized aluminum phantom, are approximately ±2% and ±2 degrees, respectively. We will be using these methods to detect possible bone mineral distribution and structural changes to disuse and exercise in conjunction with collaborative muscle studies at the Ames Research Center. Quantification of Daily Activity: Ground-contact time analysis of laboratory-collected walking and running ground reaction force (GRF) data from 23 subjects demonstrated the potential feasibility of estimating daily external loading histories from foot-ground contact time. Peak cyclic walking and running vertical GRF are estimated from group linear regression with a coefficient of variation of 3.5 %. To check feasibility outside of the laboratory, we have reprogrammed our ground reaction force-monitoring system to collect simultaneous foot-ground contact times and vertical ground reaction force. A human research study has been approved that will compare other methods of quantification with direct force measurement. We will also investigate the practicality of foot-ground contact time measurement as an alternative to direct force measurement during daily activity.

This proposal addresses both research and technical goals that NASA, the National Research Council (NRC), the National Institute of Health (NIH), and the National Institute on Aging (NIA) have identified as critical to space biology and medical science and health care on Earth. 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. 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. Bone remodeling theories and animal studies have identified peak cyclic force levels (or cyclic tissue strain energy density), number of loading cycles, and load (strain) rate as contributors to the bone modeling/remodeling stimulus. To test our mathematical model of bone apparent density and to investigate the influence of mechanical forces on bone density and whole long bone structural properties, we have focused on the calcaneus and lower limb bones as model sites loaded by muscles and joint forces that are predominantly determined by the external ground reaction force (GRF). These bone sites are also most significantly affected by long duration space flight. In keeping with the above focus, the proposal has the following objectives: (1) to validate our new method of computing long bone geometric and structural properties from densitometry and the complementary goal of establishing the dependence of long bone material and structural properties on mineral distribution; (2) to establish a

new method for quantifying physical activity level and its complementary goal of establishing a functional relationship between daily external loading histories, bone density, and long bone structural properties. Consistent with our hypotheses is the view that muscle and bone mass can be maintained in space with appropriate exercise equipment and protocols designed to provide equivalent levels of mechanical stimulus to muscle and bone tissue. We believe progress in these areas will allow us to prescribe equivalent exercise loading histories in space compared to baseline astronaut and cosmonaut activity levels on Earth, better assess fracture risk on Earth and in space, and perhaps ameliorate age-related bone loss and idiopathic osteoporosis through sensible exercise. The results will further illuminate the character of the input stimulus that regulates bone maintenance and adaptation in response to the level of daily physical activity.

Publications, Presentations, and Other Accomplishments:

Breit, G., Katz, B., and Whalen, R. "Enhancement of DXA images for determination of structural properties of small animal long bones." 11th International Bone Densitometry Workshop, Gleneden Beach, OR, September 24-28, 1995.

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Whalen, R., Katz, B., and Cleek, T.M. "Validation of long bone mechanical properties from densitometry." 11th International Bone Densitometry Workshop, Gleneden Beach, OR, September 24-28, 1995.

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."

Renal-Endocrine Response to Gravity and Sleep Disruption

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Funding:

Project Identification: 199-18-17-14
Solicitation:

Initial Funding Date: 9/94
Expiration: 8/96

FY 1995 Funding: $115,961
Students Funded Under Research: 1

Task Description:

Weightlessness during space travel has been associated with a number of changes in cardio-renal function. Its primary manifestations are a dramatic cephalad shift in body fluids leading to maladaptive hemodynamic and osmoregulatory responses, a decrease in total body fluid volume, and abnormal responses to upright posture and exercise on return to Earth. While it is clear that weightlessness produces profound changes in sodium and volume homeostasis, the mechanisms responsible for these changes are incompletely understood. In part, the ignorance is secondary to methodological problems in space. Because of this, land-based studies have attempted to unravel the mechanisms involved by using models which presumably simulate weightlessness, i.e., head-down tilt. However, substantial deficiencies exist in the presently available data. For example, 1) for the most part, studies have focused on cardiovascular rather than renal and hormonal responses; and 2) no studies have considered the impact of a disrupted circadian rhythm and/or sleep disruption as additional contributing factors. Thus, the present proposal has as its overall objective the assessment of the impact of altered gravity and disrupted sleep on renal and endocrine responsiveness in humans. To achieve this overall objective, we will evaluate renal blood flow and the status and responsiveness of the renin-angiotensin aldosterone system in both simulated microgravity and normal gravity. By assessing responses under two gravitational forces, we anticipate gaining a better understanding of the impact of weightlessness on these systems. Two specific hypotheses will be tested during this project: 1) that microgravity modifies the acute responsiveness of the renin angiotensin system, aldosterone and renal blood flow to postural changes; and 2) that chronic sleep disruption modifies the circadian rhythm of the renin-angiotensin system and its responsiveness to postural challenges. An environment simulating microgravity and sleep disruption will be used in human subjects to address these hypotheses.

It is anticipated that the combination of enforced microgravity and sleep disruption will substantially modify the acute responsiveness of the renin angiotensin system to upright posture. This, in part, will be mediated by a change in the circadian rhythm of plasma renin activity induced by the protocol. It is also likely that renal blood flow will be modified by the protocol. These responses will be correlated with the simultaneously obtained hemodynamic factors (blood pressure and pulse). It is anticipated that instability in hemodynamic factors are likely to be correlated with abnormalities in hormonal responses to the upright posture. If there are modifications in renal blood flow or the acute responsiveness of the

renin-angiotensin system, then sleep disruption could be an important variable contributing to the altered sodium and volume homeostasis associated with space flight. Twelve subjects will be used to test each of the two hypotheses outlined above. Half of the subjects will be males and half females and all will be free of any known disease. All subjects will be between the ages of 21 and 45 years of age. Individuals on an ad lib activity schedule will be placed on a 100 mEq sodium, 80 mEq potassium, 2,500 ml fluid intake daily. After equilibrating on this diet for four days, a baseline set of experiments will be performed. Individuals will undergo the supine to upright posture study on the first day, the upright to supine posture study on the second day, and a control supine study with renal blood flow on the third day. Blood sampling during the control day will be similar to that used during the supine to upright study day. On the evening of the first day of the protocol, the subjects also will have the diurnal hormonal technique performed. The subjects will then be placed at 6 degree head-down tilt for five days, while simultaneously undergoing forced desynchrony, and then the above protocol will be repeated.

The initial phase of the second protocol is identical to Protocol 1. Upon completion of the baseline studies, the patients will undergo a forced desynchrony protocol to induce sleep disruption in a normal gravity environment. At the end of five days of sleep disruption, an identical set of posture challenges will be completed. Initial efforts to implement the program as a single protocol led to several technical and logistical difficulties. Therefore, we have refined the protocol and separated it into two parts, rather than one continuous protocol. The two parts will address each of the specific aims outlined above. Thus, each phase will consist of approximately a thirteen-day admission to the Intensive Physiologic Monitoring Unit, rather than a 30-day admission. Thus, instead of 12 studies to be completed, it will be necessary to complete 24 studies, each half in time length. We anticipate completing these studies over the next 18 months, allowing an additional two to three months for analysis and modest follow-up on questions generated during the principal phase of this study. Beginning January 1, 1996, this will require the hospitalization of two subjects each month. We are presently on target in achieving this goal.

This research is primarily directed toward gaining a new understanding of basic biologic processes. Weightlessness during space travel has been associated with a number of changes in cardio-renal function. Its primary manifestations are dramatic cephalad shift in body fluids, leading to maladaptive hemodynamic and osmoregulatory responses, a decrease in total body fluid volume, and abnormal responses to upright posture and exercise on return to Earth. It is unclear based on the present information how much of these maladaptations may be due to weightlessness and how much are related to the disrupted circadian rhythm that accompanies travel in space.

The changes observed are similar to the changes that have been proposed as underlying the pathophysiology of some individuals with edema disorders and hypertension. Thus, the information obtained from these studies could be applicable also to understanding part of the pathophysiology of these common conditions. With this understanding, a better approach to treatment of these conditions will be possible.

Biochemical Changes of Bone in a Model of Weightlessness

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-26-17-06

Solicitation:

Initial Funding Date: 5/94

Expiration: 5/97

FY 1995 Funding: $134,000

Students Funded Under Research: 1

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 it 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.

The mineralizing turkey tendon has been used as a model for a study on the relationship between collagen structure and mineralization. The peripheral-(Peri-- never-mineralized), inner mineralized-(Mineral.) and inner nonmineralized (Nonmin.) collagens were separated from leg tendons and subjected to cross-link analyses. In the case of 57 week old animals, both Mineral. and Nonmin. collagens demonstrated the major cross-link precursor to be hydroxylysine aldehyde (>80%). Most of them were

converted to pyridinoline (Nonmin.) or pyridinoline and deoxy-pyridinoline (Mineral.). In Peri., however, the major cross-link precursor was lysine aldehyde (>70%) leading to the formation of dehydro-histidinohydroxymerodesmosine. These data demonstrate that Peri collagen has a post-translational chemistry different from either of the other two collagens. In addition, using 12, 17 and 33 wk old turkeys, we dissected the tendon into 6 portions (from ankle to muscle site) and analyzed for cross-links. In the mineralizing area of tendon, the content of pyridinoline diminished (0.82 mole/mole of collagen in 12 wk to 0.3 in 33 wk old) but deoxypyridinoline increased (0.02 in 12 wk to 0.40 in 33 wk old) as mineralization proceeds. These data indicate that collagen deposited prior to/during mineralization is post-translationally distinct from that of the nonmineralized portion. The potential regulatory roles of cross-linking in mineralization and in the process of bone loss will be further pursued by determining their molecular distribution in the fibril.

We also have studied bone collagen cross-links in the toothless (tl/tl) osteopetrotic rats and compared them to those of normal healthy littermates and tl/tl mutants after treatment with colony stimulation factor-1 (CSF-1). It was found that the concentration of pyridinoline and deoxypyridinoline cross-links in untreated tl/tl mutants were 2-4 times higher than in normal healthy littermates. These values, however, were clearly diminished in tl/tl mutants treated with CSF-1; although the values were still somewhat higher than in control. In addition, we found that the cross-linking chemistry in long bone collagen is clearly different from that of parietal bone. Recently, in collaboration with Dr. Masse, Univ. Moncton, Canada, we have characterized collagen cross-links in cartilage and tendon obtained from vitamin B6-deficient chicks. In addition, it was found that bone mechanical properties of B6-deficient animals were impaired. Our preliminary studies on bone collagen cross-links showed that dehydro-dihydroxylysino-norleucine was significantly increased in B6-deficient bone indicating an increase in newly synthesized collagen. This is consistent with our previous findings in primate disuse osteoporosis.

In another study, we evaluated the effect of treatment with Cl2MBP on bone remodeling during immobilization in rats. Male Sprague-Dawley rats were divided into three groups. Animals in two groups (with and without treatment with Cl2MBP) were subjected to one hindlimb immobilization. The third group was control (nonimmobilized). In immobilized vehicle-treated animals, a significant decrease in femur strength (by 8%), an increase in ductility (by 16%) and a slight decrease in mineral content were observed when compared to the control. However, in the immobilized-Cl2MBP-treated group, a significant increase in femur strength (by 10%) and an increase in mineral content (by 8%) were confirmed. In addition, there was a significant decrease in trabecular bone volume (TBV) (by 15%) in tibia from the immobilized vehicle-treated group, while an increase in TBV (by 50%) was observed in the immobilized Cl2MBP-treated group. These data indicate that the treatment alter the mechanical properties of immobilized femurs. Many other studies were partially supported by this grant in order to elucidate the collagen structure and its interaction with other matrix components in relation to mineralization.

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 these disordered mineralization.

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 multilfold from a basic understanding of the collagen fibril structure, mechanisms of bone mineralization and bone loss, to a clinical application.

Publications, Presentations, and Other Accomplishments:

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Visual Vestibular Interaction

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Funding:

Project Identification: 199-16-17-07
Solicitation: 93-OLMSA-07

Initial Funding Date: 3/94
Expiration: 3/97

FY 1995 Funding: $330,000
Students Funded Under Research: 8

Task Description:

This grant funds a consolidation of three research projects:

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.

2) Subjective Responses to Linear Acceleration and Haptic Stimulation (L. R. Young)
The overall objective of projects two and three 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 of project two is on linear and angular acceleration stimuli, coupled to optokinetic vection stimuli; we will also increasingly encompass inputs gained from the companion studies on other modalities, specifically the roles of haptic (tactile and propioceptive) cues, mental sets, learning and experience, and active versus passive control.

3) Oculomotor and Postural Responses to Linear Acceleration (L. R. Young)
In project three, human oculomotor and postural responses to linear acceleration will be addressed in three different studies. In the first, linear visual vestibular interaction on the MIT "sled", the binocular eye movements of human subjects during sinusoidal (0.5 g, 0.25 Hz) linear acceleration aligned with the subjects' inter-aural axis in conjunction with linear motion of a visual display. In the second, influence of lateral linear acceleration on ongoing horizontal OKN, horizontal and vertical eye movements will be recorded in human subjects during inter-aural and rosto-caudal sinusoidal linear acceleration. The third study is otolithic contributions to torsional eye movements during dynamic linear acceleration, using the sled apparatus and the ISCAN facility.

For project 1, a preliminary series of simple but important experiments addressing figure recognition were completed, exploring the effect of retinal versus gravitational orientation on the recognition of three different types of figures. A low cost Virtual Environment system is being developed for use in the human visual orientation research. Experiments employing the VE system to create a virtual "tumbling room" are being designed, to test the hypothesis that visual reorientation illusions similar to

those experienced in weightlessness can also be created in supine subjects when a virtual environment is rotated in the frontal (roll) plane about an Earth vertical axis.

In project 2, to test the hypothesis that otolith information may be used for recreating a path in space as well as for detecting linear motion, data analysis protocols have been developed and our sled facility prepared for this experiment. We are also evaluating the role that tactile cues play in the perception of motion through the use of the NASA Langley "G-seat" in experiments related to human perception of whole-body motion.

For project 3, study one, we found that the oscillatory component of the horizontal response to sled motion did not depend on the orientation of the optokinetic display. Further, no consistent oscillations were observed in the vertical response, even when the optokinetic stimulation was vertical. These results suggest that the oscillatory response is primarily due to a summation of a linear VOR with the optokinetic response and not to a modulation of the optokinetic response by the linear acceleration.

In study two, in all conditions the response of subjects at the lower frequency was less than that at the higher frequency and the response during z-axis was less than in y-axis. This result is predicted by a sensory conflict model which predicts that at the lower frequencies the visual system would dominate and at the higher frequencies the vestibular response would inhibit the visual.

In study three, it was concluded that OT is primarily elicited in the tested frequency range by y-axis acceleration, hence by stimulation primarily of the utricular organs and that response amplitude and phase shift cannot be fully explained by peripheral mechanisms, but that central influences must also be considered.

Molecular Damage of Human Cells by X-rays and Neutrons

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Funding:

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Students Funded Under Research: 7
Joint 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. DNA damage induced by fission neutrons will be compared to that induced by isoeffective 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 phase 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 15 days. We will study gene expression at several time points within the first three weeks post-irradiation using a variety of established or novel techniques presently used in our laboratories for studying molecular changes associated with human malignant disease. Types of DNA damage to be studied include induced microsatellite instability within multiple loci, mutations in several cancer-related genes, and mutations in novel gene segments utilizing newly-developed polymerase chain reaction (PCR)-based methods. In addition, we will assess permanent alterations in the expression levels of both known and novel gene transcripts, using reverse transcriptase (RT)-PCR, Northern blotting, and a novel differential gene expression assay. In the proposed studies, reactor-produced neutrons are considered to be a useful surrogate for HZE particles. A sizable proportion of the dose absorbed behind spacecraft or lunar shielding is due to neutrons. In terms of resources, this project is a joint effort between the University of Maryland School of Medicine and the Armed Forces Radiobiology Research Institute.

The overall objective of our program is to characterize in molecular terms genetic alterations induced by ionizing radiation. Our FY95 specific aims in pursuit of this goal include: (1) to complete our study of selected genes involved in repair and/or cell cycle regulation; (2) to begin differential gene expression studies with low-LET radiation; (3) to begin studies of site-specific genomic instabilities on chromosome 13 and 17.

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. Our efforts focused on supporting an exciting new finding that certain proteins involved in DNA replication and repair of low-LET radiation damage are altered in cellular survivors, and that this alteration depends on the pre- existing status of the p53 tumor suppressor gene in these cells. We found that one of the genes regulated by p53, wild-type p53-activated fragment 1 (WAF1/Cip1), has a bi-phasic induction pattern; that is, its induction in cells with the normal p53 gene first peaks within several hours after X-irradiation, then declines, and peaks again 4 to 7 days after X-irradiation. This finding provides a possible mechanism for alteration of proteins comprising DNA replication/repair complex, since the proteins comprising this complex are regulated by the p53/WAF1 genes. Independent of the above experiments, we have initiated an assay for screening differentially expressed genes in X irradiated versus control cells. To date, we screened 1400 genes from a commercial cDNA library (from HL60 cells), and found two candidate genes differentially expressed cells irradiated at 4 Gy X-rays, 3 h postirradiation. Finally, we have begun a feasibility study on mutation detection in short-repeat DNA sequences (termed micro-satellites) flanking the chromosomal location of the p53 gene. We used two cell lines differing in the p53 gene status as a model system. Although we were able to detect differences in DNA length in these regions between the two cell lines, we did not detect any differences in micro-satellites between X-irradiated and control cells.

Conceptually, one of the most challenging tasks in this project has been to demonstrate a feasibility of studying gene expression in surviving cells without any additional phenotypic selection. Common approaches to selecting cells for molecular radiation biology experiments include comparing tumor versus normal tissues (cells), or locus-specific mutants versus normal cells. However, since these phenotypic changes are the result of accumulating multiple genetic sequential changes in the cell, they are generally disease-specific rather than agent-specific. In contrast, our approach assumes that a molecular radiation signature can be detected during the early stages of phenotypic evaluation before any phenotypic markers are apparent. We validated our approach by demonstrating differential expression of specific genes participating in DNA replication/repair.

A practical rather than conceptual problem has arisen in relation to the proposed studies of radiation-induced instability in micro-satellite regions of the genome. The negative results for irradiated and control cells suggested to us that the assay is not sensitive enough to detect changes in DNA length in a limited number of affected cells, if the pooled population of affected and unaffected cells is studied. Two approaches to improving the assay sensitivity are being considered. The first solution is to analyze DNA from clonal populations, that is, populations established from a single cell. This approach would entail establishing 50-100 single-cell populations per locus per exposure condition. In another completed study, we worked out a protocol for establishing clonal populations from a single cell (by assuming that the number of cells/well is Poisson distributed with the mean = 0.5). In the context of the present task, this time/effort intensive approach is not considered a viable solution given the limited resources of this laboratory. Alternative approach is to perform the micro-satellite assay using multiple samples of extremely diluted DNA solution such that DNA concentration would be comparable to DNA concentration in a single cell. Although this approach does not eliminate the necessity of performing multiple assays for the same locus, it does eliminate the necessity of establishing multiple cl onal populations for the same exposure conditions, a substantial saving in terms of our resources.

Since we have already validated our approach by detecting specific molecular alterations in X-ray-survivors, it is important to address our second hypothesis that differential alterations result from high-LET compared to low LET radiation by performing similar experiments using cells exposed to high-LET radiation. The proposed high-LET radiation source was the Armed Forces Radiobiology Research Institute (AFRRI) reactor in Bethesda, MD. Cryopreserved cells, previously exposed to fission neutrons, are available for such studies. New irradiations at the AFRRI Reactor facility are planned for the spring 1996.

This research seeks to understand at the molecular level the biological consequences of exposure to increased radioactive background. Recent investigations demonstrated that A-bomb survivors had increased rates of chromosomal aberrations (reciprocal translocations), somatic mutations, elevated risk of leukemia, breast and lung tumors. Also, studies carried out in areas contaminated after the Chernobyl disaster allow definite conclusions that complex changes take place in animal and man in an altered radioecological situation. These changes include disorders in immune and hemopoietic systems, gastrointestinal tract, development atheriosclerosis, 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 a man is exposed to. 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 allow to delineate the expression and mutation profile of specific genes that are differentially regulated in radiogenic cancer and other late effects of radiation.

The conversion of a normal cell into an abnormal cell is largely the result of change in gene expression patterns between 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 being currently developed for other specific human populations at risk, for example, of developing breast or colorectal cancers or mental disorders due to genetic hereditary factors.

One potential advantage emerging from our studies is the possibility of modelling 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. One exceptional situation on Earth has developed as a result of the Chernobyl accident. A large number of people and their offspring have been, and will continue to be, exposed to a broad spectrum of radionuclides including a-, ß- and g-emitters; this situation will persist for protracted durations due to 137Cs and 90Sr 30-year half-life and more than 10,000 year that of plutonium. 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.

Despite the fact that ionizing radiation is the most regulated substance on Earth, and the fact that ionizing radiation is a weak carcinogen compared to other harmful agents human populations are exposed to, there is a more defined public perception of risk from the exposure to ionizing radiation than from these other agents, including ultraviolet light and environmental chemicals. This fear is fueled in part by common knowledge of tragic and complex consequences to human health following the Chernobyl accident and detonation of nuclear weapons over Hiroshima and Nagasaki. However, a contributing factor is the gap in our knowledge concerning the molecular mechanisms of radiation action at the cellular level so that most of biological effects of radiation cannot effectively be communicated to the general public. In a therapy setting, a relevant radiation action can be explained to patients by comparing radiation to a "pacman" that "eats" cancer cells. Such a cartoon representation can be made because the scientific basis of radiation therapy are well-understood.

The technologies we are developing could be termed *differential radiation molecular biology*, since they provide sensitive molecular measures of changes specifically by induced by different types of irradiation, help to define the nature of genetic lesions and help to analyze the action of specific genes in health and disease. Although our studies are limited to investigating radiation effects, they could be applied for detecting changes due to any other agents, including medically important DNA-reactive drugs. As described in *Progress Report*, one method will provide information about the chromosomal breakage sites and structural rearrangements within individual chromosomes. The second method is a strategy for screening a very large number of possible gene targets regardless the normal level of transcription in the cell. This method consists of preferential screening of HL60 cDNA library. The primary cDNA library have been obtained from a commercial source. The Stratagene Lambda *ZAP* system have been used for the secondary library construction. It provides a simple method for obtaining plasmid DNA clones from the original phage isolates. The strength of our method lays in the fact that individual randomly selected cDNA phage isolates are used. Our protocol permits screening of 200 plasmid cDNA clones per procedure. Namely, two identical agarose gels are prepared with each lane containing a mixture of 10 polymerase chain reaction (PCR)-amplified cDNA inserts (thus, a gel containing 20 lanes with each lanes containing 10 amplified inserts posses 200 inserts) and Southern blots are made from each gel. DNA hybridization probes are prepared, one of each from control-cell and treated-cell mRNA and reverse transcriptase reaction. Thus, the method can be used to identify expressed genes in any two different mRNA samples, and could potentially displace other types of molecular analyses used for purposes of comparing two tissues (or two cell samples).

Publications, Presentations, and Other Accomplishments:

Balcer-Kubiczek, E.K., L. Malkas, S. J. Meltzer, and G.H. Harrison "Stable alteration of DNA replication complex in X-ray survivors." Radiation Research Congress Proceedings, Abstract P27/20, vol 1, 393 (1995).

Lens Epithelium and Proton-Induced Cataractogenesis

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No Co-I's Assigned to this Task

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Task Description:

Cataracts are a potential late side effect of space travel that impacts risk assessment and space craft 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 an 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 xray-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.

The major accomplishments of our work to date have been the characterization of the morphological and functional stages of the maturation of the human neonatal lens epithelial cells in culture. This is in preparation for our x-ray and proton studies. We believe that our system is uniquely demonstrating normal differentiation in vitro of lens epithelial cells into mature lens fiber cells, based on measurement in confluent HLE cells of significant levels of the gamma-crystallin protein which is a

marker for the lens fiber cell. Exponentially growing HLE (3 days after plating) show evidence for the presence of alpha- and beta-crystallins, and only a low level of gamma-crystallin protein. We also have preliminary evidence for radiation-induction of bFGF in the HLE cells. In summary, we have made significant progress during our first year of funding to establish the required baseline growth, and differentiation endpoints and to complete some of the pilot x-ray and proton experiments for our proposed human lens epithelial cell studies. Our plans for the coming grant year do not deviate substantially from our original proposal. The 88" accelerator schedule will allow us to continue our experimental schedule as planned. During the next funding year we expect to finish the proposed survival and micronuclei studies and begin the chromatin breakage and repair work. The successful progress in our measurements of both bFGF mRNA and protein during the past year will allow completion during the second year of a major portion of the studies to examine the time course of bFGF modulation by radiation. Finally our successful measurements of lens crystallin protein profiles during the past year now allow the study of x-ray and proton effects.

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 what are 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 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 threshold differ significantly from zero, but both are much less that 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 Fibroblast Growth Factor (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 counter-measures 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.

Publications, Presentations, and Other Accomplishments:

Blakely, E.A., Bjornstad, K.A., Polansky, J.R., and Lui, G.M. "Characterization of neonatal human lens eipithelial cells grown on ECM (Abstract of a poster)." 1995 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL, May 14-19, 1995.

Proton Radiation Studies

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-45-17-04

Solicitation:

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Expiration: 6/97

FY 1995 Funding: $ 200,000

Students Funded Under Research: 0

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-two 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 is being asked to support the surviving monkeys and the program for at least 3 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, 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.

The long-term project, "Proton Radiation Studies" is now in its 32nd year, and 20 out of the original 358 monkeys in the project are still alive as of the end of this fiscal year. A majority of the survivors (13/20) are subjects irradiated in 1969 (and their age-matched controls). The 1969 group is critical to the experiment because these individuals were exposed to "simulated solar flare" protons (a mixture of

10- and 110-MeV protons) to mimic the most likely exposures to which astronauts might be exposed. We expect these animals to last 3-4 more years, and data on all late radiation sequelae in these monkeys will be among the most important we will obtain.

In addition to the semiannual physical examinations and extensive histopathological workups accomplished when each animal reaches the end of its life span, tissues are being collected from all the subjects and frozen in liquid nitrogen for future analyses. Advances in molecular genetics are occurring so rapidly that we hope to utilize the tissues for determinations of, e.g., oncogene(s) and senescence gene(s) expression. The pathologists continue to see intestinal tumors in many of the oldest subjects. Colon tumors appear primarily in controls, while tumors of the duodenum and ileum appear in the irradiated animals. We are planning to compare our small data base on intestinal tumors with the much larger data base on the survivors of the Hiroshima bomb in 1995, including controls, as well as other human populations. Heart disease is common in our aged subjects, both irradiated and control. The rhesus monkey model is an excellent one not only for late radiation effects studies, but also for aging studies. The radiogenic cataracts we have monitored in the monkeys for over 10 years will allow us to perform extrapolations from other animal models to the nonhuman primates and to extrapolate data from the monkeys to humans.

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 the common man, and, importantly, on the common woman. 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 this year, 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 standard laboratory rodents.

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 data base 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.

Since 1986, Dr Cox has been discussing 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 hope to start working with the data there, supported by NASA, during 1996. 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 were able to convince the scientists and physicians at the RERF that they should 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 the funding for the 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 boon to that particular population.

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 NASA-supported research on the aging/irradiated monkeys will inspire new emphasis on nonhuman primate models for debilitating diseases in humans.

After Dr. Cox presented a seminar on her NASA project to students at the University of Texas at El Paso (UTEP) in January 1995, she was approached by Prof. Sid Das of the Biology Department for names of NASA contacts willing to participate in a Space Day at UTEP. Since the Biology Department at UTEP is part of an NIH program called Minority Access to Research Careers (MARC), Dr. Cox approached Dr. Gary Coulter at NASA Headquarters for suggestions, and he contacted Prof. Das directly. Together they developed a program for the UTEP students sponsored by the MARC program. Space day is to be held on March 6, 1996. Topics to be covered include the Space Station (incorporating linkups with Mir), access to SLS data archives, Immunology in Space, and Training Opportunities. This contribution by NASA to an outstanding Minority Training institution is to be commended.

Publications, Presentations, and Other Accomplishments:

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Human Enzymatic Repair of Radiation-Induced DNA Breaks

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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 of 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 have 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 is largely determined by the type of strand break on the plasmid (i.e. single versus 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 a DNA damage, biological outcome should the same regardless of the type of radiation (eg. HZE vs. x-rays) that produced the lesion. Thus, lesion production becomes the primary predictor of the biological endpoint; and their yield can be assessed directly as a form of biological dosimeter. To confirm this hypothesis we have irradiated shuttle vector plasmid with high energy 57Fe particles at the Alternating Gradient Synchrotron at Brookhaven National Laboratory and hope to compare their mutagenic potential of this DNA with gamma irradiated and bleomycin treated DNA.

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. Rodent fibroblast lines SCID and xrs-5 are sensitive to ionizing radiation and deficient in the proteins involved in DNA double-strand-break repair. The SCID and xrs-5 cells contain a defect in the DNA-dependent protein kinase (DNA-PK) and the p70 subunit of the Ku heterodimer, respectively. However, the fidelity of DNA double-strand-break repair is unknown in these cell lines. We tested the ability of these cells to correctly repair double-strand breaks generated by the radiomimetic drug, bleomycin, using a shuttle vector mutagenesis assay system. Our results indicate that SCID and xrs-5 cell lines have two-fold higher mutagenesis in repairing double-strand breaks than parental wild-type controls -- a difference comparable to their two-fold cellular radiosensitivity. The fidelity of double-strand-break repair was also 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-PK. The A-T cells show a 2 to 3.4-fold increase in mutagenesis compared to the normal fibroblast cell line, MRC-5. 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.

The relative role of human DNA polymerases in the DNA strand-break repair process. Human nuclear polymerase utilization for the repair of a major class of ionizing radiation-induced DNA lesion -- DNA strand breaks containing 3'-PG -- was examined using a novel, chemically-defined, vector substrate containing a single, site-specific, 3'-PG-containing single-strand-break lesion. In addition, the major human AP endonuclease, HAP1 (APE1, APEX, Ref-1) was tested to determine if it is involved in initiating repair of 3'-PG-containing single-strand break lesions.

Nuclear extracts of human HeLa S3 cells were prepared, and alpha, beta, gamma, and epsilon polymerase activities were identifies and quantitated based upon their differential sensitivities to BuPdGTP, aphidicolin, ddTTP, and a requirement for PCNA. Extracts which had been selectively inhibited for all but one of the polymerase activities were used in repair reactions with an M13 vector construct possessing a site-specific single-strand break lesion with the exact chemistry and configuration as the authentic ionizing-radiation-induced DNA single-strand-break lesion.

DNA polymerase beta was found to be primarily responsible for nucleotide incorporation at the lesion site following 3'-PG excision. Also, it was demonstrated that the 3'-PG excision repair initiation reaction, was not dependent upon HAP1 activity as judged by immunoprecipitation and inhibition of HAP1, with neutralizing HAP1-specific antibody.

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 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.

Publications, Presentations, and Other Accomplishments:

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Mutations in Human Lymphoid Cells

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FY 1995 Funding: $206,844
Students Funded Under Research: 4

Task Description:

The goals of this proposal are to determine the susceptibility of human cells to mutagenesis following low dose 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.

One emphasis in the first year has been quantitation of the risk of proton-induced mutation in two human lymphoblastoid cell lines derived from the same donor. These cell lines have different susceptibilities to 55 MeV proton-induced cytotoxicity and mutagenesis for the autosomal, heterozygous thymidine kinase (tk) locus and the X-linked, hemizygous hypoxanthine phosphoribosyltransferase (hprt) locus. Our very preliminary characterization (using a gel mobility shift assay) suggests that radiosensitive TK6 cells are wild-type for the tumor suppressor gene p53. WTK1 cells are more resistant to proton-induced cytotoxicity and mutagenesis. It has been recently reported that the radioresistance of WTK1 cells may be due to a homozygous p53 mutation. Thus, susceptibility to particle-induced mutations may be affected by the genetic constitution of individual target cells. A proton or Fe ion traversal of a cell with normal p53 may result in cell death more often than in a cell carrying a pre-existing mutation in p53. Furthermore, cells with p53 mutations may be intrinsically unstable. Inherent instability may permit rapid evolution within clones that survive particle irradiations. The increased evolutionary rate may be associated with an increased carcinogenic potential based on the cellular genotype.

We continued our studies of tk and hprt mutant spectra associated with low fluence exposures to Fe ions. We analyzed 131 mutants selected from cultures exposed to <3 particles per cell of Fe ions. Large deletions were the most common mutations observed at either locus. We are evaluating the

extent of these deletions by testing for loss or retention of other loci on the same chromosome arm. No tk-ng mutants examined thus far have lost flanking markers on chromosome 17q. Deletions as large as 2 Mb occur on chromosome Xq, inclusive of the hprt gene, but most mutations extend less than 350 kb on either side of hprt. We have collected > 200 hprt and tk mutants from cultures exposed to 55 MeV protons and expect to characterize those mutations in the coming year. We plan to focus next on 1) susceptibility of TK6 and WTK1 cells to 1 GeV Fe-induced mutation, and 2) collection and characterization of mutants isolated after low dose exposures to protons or Fe ions.

The progress toward our goals has been essentially as planned in the initial application. We have made substantial progress on the variation in response of different endogenous genetic loci to mutation induction by protons and Fe ions. We have begun to characterize the difference in susceptibility to mutation of the same genetic locus in syngeneic cell lines that differ in expression of the tumor suppressor gene p53. Our preliminary studies suggest that the cell line that expresses normal p53 is less readily mutated by protons or Fe ions as compared with the cell line that expresses mutant p53. This finding highlights the possibility of distinct susceptibilities of cells originating within the same individual to mutation induction by energetic protons or heavy ions that exist in the space radiation environment. We have made substantial progress in the molecular characterization of mutations induced by low fluence exposures to energetic Fe ions, and have assembled a large collection of proton-induced mutants for future analysis. In addition, we have assisted in the development of a new facility for NASA sponsored high energy heavy ion research at the Alternating Gradient Synchrotron at Brookhaven National Laboratory, another low energy facility (the 88 inch cyclotron) at Lawrence Berkeley National Laboratory, and we carried out experiments at the Loma Linda University proton accelerator.

Implementation of Proton Studies at the 88 inch Cyclotron - Initial energy 55 MeV/amu. We participated in the establishment of a new biology beam line at the 88 inch cyclotron at Lawrence Berkeley Laboratory. Physical characterization of the proton beam was carried out with the assistance of Cary Zeitlin, Lawrence Heilbronn, Jack Miller, Peggy MacMahan and William Holley (all at LBL). We also established dose-response relationships for proton-induced cell killing and mutation in TK6 cells using this beamline. More than 200 hprt or tk-deficient mutants have been archived for molecular analysis. Cell killing did not differ from what we have observed for 150 kVp X-rays. Mutation frequencies are low but detectable, and are in the range of our X-ray results.

Preparations for 1 GeV/amu Fe irradiations at the Alternating Gradient Synchrotron at Brookhaven National Laboratory. Effort was given to the preparation and coordination of aspects of the multi-investigator proposal to perform experiments with 1 GeV Fe ions at the Alternating Gradient Synchrotron facility at Brookhaven National Laboratory (Jack Miller, Gregory Nelson and Amy Kronenberg, spokespersons). Extensive interaction with the Brookhaven staff has been required to specify beam line requirements and to design the biology support facilities within the AGS complex. We anticipate taking data on the new biology beam line in September 1995.

Feasibility Studies with syngenetic cell lines with different genotypes for the p53 tumor suppressor gene. We established the use of the WTK1 cell line (a gift from Dr. Howard Liber) for future use in low fluence Fe experiments. The WTK1 cell line is derived from the same original donor as the TK6 cell line. Both cell lines are hemizygous for the hprt locus and are heterozygous for the tk locus. The active tk allele is found on the same copy of chromosome 17 in both cell lines. TK6 and WTK1 cells differ in radiation response (Amundson and Liber, 1991). The TK6 cells are known to be radiosensitive and are less readily mutated than are the WTK1 cells. It has recently been reported that WTK1 cells are homozygous for a mutation in exon 7 of the p53 gene, while TK6 cells have normal p53 alleles (Xia, et al., 1995). Studies using 55 MeV protons showed that WTK1 cells are more readily mutated than TK6 cells. Initial studies using WTK1 cells for studies at Brookhaven demonstrated that cells carrying a pre-existing mutation in p53 are more susceptible to mutation induction by energetic Fe ions than their syngenetic counterparts with normal p53.

Molecular Characterization of Mutations Induced by Low Fluence Exposures to Fe ions. We continued the characterization of both hprt and tk-deficient mutants of human TK6 cells isolated after low fluence exposures to 600 MeV/amu Fe ions. Intragenic analysis for the hprt locus was performed using both Southern blotting and multiplex PCR techniques. Linked marker analysis was initiated using a series of PCR primers and Southern hybridization for sequences at known physical distances from the hprt locus. The hprt mutants are largely deletion mutations and such deletions range from a few basepairs to up to perhaps 2 million basepairs. Our mapping studies at the tk locus show that loss of heterozygosity is common. In contrast to results obtained with other charged particles with lower LET's, fewer slowly growing allele loss mutants are observed after these exposures. The mutants characterized thus far have not lost either of the VNTR markers (D17S24 and D27S74) located on chromosome 17q. We plan to analyze these mutants further using a series of microsatellite repeat probes within the region bounded by D17S24 and D17S74 to determine the extent of loss in both the normal growth and slow growth tk mutants isolated after low fluence Fe ion exposures. Taken together, these data suggest that deletions or allele loss of up to several million basepairs may result from a single Fe ion traversal provided the region of interest on the chromosome can tolerate such losses.

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.

Publications, Presentations, and Other Accomplishments:

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Radiobiological Studies - Task V (Final Year)

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Funding:

Project Identification: 199-45-17-07

Solicitation:

Initial Funding Date: 7/95

Expiration: 6/96

FY 1995 Funding: $90,000

Students Funded Under Research: 2

Task Description:

The project will be completed as planned. Currently, surviving animals will be scored for cataracts until they become moribund, or are otherwise recommended for euthanasia by attending veterinarians in the Painter Animal Facility at Colorado State University. One, final, cross-calibration of lenticular opacification will be conducted. Analyses of DNA damage in photoreceptor cells and their losses from the retinas of sacrificed animals will be conducted as previously. Overall analyses of the changes in the size distributions etc. of the photoreceptor DNA will be conducted when all the DNA sedimentation profiles have been obtained. Final reports will be prepared, and articles written, in time for the 31st COSPAR Scientific Assembly in Birmingham, U.K. in 1996.

If time and funds permit, preliminary efforts will begin at extrapolation across species (rats, rabbits, dogs, monkeys to humans) for radiation cataractogenesis using data collected over 25 years, e.g., see reference 1. These efforts will be concomitant with, and complementary to, the project at RERF, Hiroshima, should that research proposal be successful.

For *circa* twenty-five years, a ground-based research program, which simulates biological effects in astronauts from exposure to the densely ionizing radiations that comprise the "ambient" galactic heavy-ion spectrum in space, has been conducted with an animal model, the rabbit, and representative radiations generated by particle accelerators. Whenever possible, these studies have been integrated with concurrent investigations in other laboratories, both nationally and internationally, with different animal models, especially non-human primates, and other radiations, especially protons. In this way, it will become possible to achieve an "extrapolation across species to humans" of sequelae observed with long-lived mammalian species rather than just short-lived rodents.

Currently, the last year of the last experiment in the overall program, which simulates mature astronauts with mature animals and evaluates the effect of age at the time of exposure, is approaching completion. Although the experiments still are not complete at this time, the following conclusions seem likely to be reached: 1) The induction of cataracts from exposure to the "ambient" fluxes of galactic heavy ions on extended space missions, e.g. to Mars, will be of marginal medical significance for astronauts at least until very late in their life times long after their mission careers are over. At such times, the radiation damage could be compounded by senile cataractogenesis. 2) On extended

missions beyond the protection of the terrestrial magnetosphere, damage along and around the trajectories of heavy charged particles through organized tissues of the central nervous systems could cause not only loss of "neuronal" function in astronauts late in life but also could affect performance during the missions.

This is the final year of a ground-based research program conducted over the past twenty-five years. Biological effects of the types expected to be caused in astronauts by the fluxes of heavy charged particles that will be encountered during extended space missions beyond the protection of the earth's magnetosphere were simulated with an animal model, the rabbit, exposed to beams of relativistic atomic nuclei generated by a particle accelerator. The overall research program sought primarily to provide: 1) Information that will improve the evaluation of the risks of late radiation-induced cataracts (lenticular opacifications) from extended missions in space. The expectation is that this objective will be achieved. 2) An evaluation of damage incurred in the DNA of retinal photoreceptor cells by galactic radiations, and its subsequent fate *in situ* throughout the lifespan of the animal model. The expectation is that this objective will be achieved. Furthermore, 3) Since the retina is considered to be a "mini-brain", it served in this program as a model for the brain (central nervous system). 4) Since the base-line experiments involved a study of DNA damage arising in retinal photoreceptor cells during natural aging, and during aging following exposure to radiation of the types encountered on earth, the research program is directly pertinent to the human situation on earth in terms of: the natural aging of the central nervous system; the possible effects of terrestrial environmental radiation on the central nervous system; the consequences of damage to the central nervous system following cancer radiation therapy. Indeed, early experiments in the program were funded by the (then) National Institute of Neurological Diseases and Stroke and the National Institute on Aging. 5) From the standpoint of basic (molecular) biological progresses, this research examined natural and radiation effects *in situ* in a terminally differentiated, post-mitotic, population of cells in the retina and, by implication, other such cell populations in the central nervous system.

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Molecular Analysis of HZE Damage in Transgenic Mice

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Funding:

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Students Funded Under Research: 0

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 meditation 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 t and a particles.

We propose 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 to recover and quantify radiation-induced mutations readily, to determine the nature of these mutations by restriction fragment length polymorphisms (RFLP), to investigate the molecular mechanisms of mutation induction by DNA sequence analysis of recovered mutants, and to assess whether there are tissue-specific mutagenic mechanisms induce by HZE radiation, especially in nondividing tissues, 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 predispose to cancer.

In the eight months since the start of this grant, our efforts have been primarily directed to the establishment of the transgene assay. This has involved determining: 1) the appropriate conditions for the isolation of genomic DNA from different tissues of the mouse; 2) the techniques for the isolation and recovery of the transgene from the genomic DNA using an antibody/magnetic bead system and

3) establishing the selectable screening procedures for the identification and recovery of the mutant transgenes. We now have these techniques established in our laboratory, although the selectable screening system will probably require some further refinements (modification of media and growth conditions).

In October we exposed 30 transgenic mice to a single 1 Gy dose of 1GeV iron ions using the Alternating Gradient Synchrotron apparatus at Brookhaven National Laboratories, Upton, New York. The mice were transported to our laboratory and are being sacrificed in groups of six at 1, 2, 4, 8 and 16 weeks post-irradiation time points. The last time point will occur on January 29, 1996. Control mice were transported to the facility at Brookhaven but were not irradiated and are being sacrificed at similar times.

Our very preliminary data indicates that there is no significant increase in the mutation frequency induced in the transgenes isolated from the livers of exposed mice sacrificed at one and two weeks post-irradiation. This is in distinct contrast to the induction of chromosome aberrations in the circulating lymphocytes of these animals. For this assay, performed in collaboration with Dr. James Tucker, Lawrence Livermore National Laboratory, aberrations were scored by the fluorescent in situ hybridization technique using mouse-specific chromosome probes. There was an approximately 100-fold increase in the frequency of aberrations at one week which had declined to 20-fold at eight weeks. This indicates that exposure to this dose of iron ions caused considerable damage to the cells, that the level of damage declines with time (although we need later time points to determine if it will return to background levels), that damage processing may occur differently in terminally differentiated circulating cells than in repopulating tissues such as liver, and that mutation induction does not necessarily follow the same pattern as chromosome aberration induction.

These results show that the system we are using, which has not been used for studies such as these before, should be able to answer many of the questions that need to be addressed. They have also revealed that we will need to use lower doses, sacrifice the mice at both earlier and later time points, and that we need to ascertain whether these results are specific for HZE particles or hold for ionizing radiations of different qualities.

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 in 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 with that in exposed human populations. The benefits that may result from this research should also impact the common man. 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.

The Effect of Single Particle Traversals on a Mechanism of Cell-Cycle Regulation

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Funding:

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FY 1995 Funding: $174,126

Students Funded Under Research: 4

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. 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 make up one or more components of the multistep process of carcinogenesis.

In work done leading up to the present project, I had recently shown that radiation-induced G2 arrest after moderate doses of x-rays (2.0 Gy) or alpha particles (0.2 G) is characterized by the transient failure of the cdc2-cyclin B1 complex to dephosphorylate and thus become active. Both cyclin B1 and cdc2 proteins are present in abundance, as are the wee1 kinase and cdc25 phosphatase which together specifically regulate the phosphorylation state of cdc2. This work was published in September of 1995.

In the present work, my hypothesis is that the radiation-induced delay in cdc2 activation may cause, or is an effect of, alteration of the normal cellular compartmentalization of the proteins. We are testing this idea in HeLa cells by observing subcellular localization of cyclin B1, cdc25, and wee1 as a function of age in cell cycle and as a function of x-ray and alpha-particle dose. All measurements are done on a cell-by-cell basis, making multiple measurements per cell. The location and abundance of the protein is measured with a confocal laser scanning microscope, as is the amount of propidium-iodide-stained DNA, which determines cycle-age. This year we have concentrated on optimizing our assay conditions. We have used immunocytochemistry to visualize cyclin B1 and cdc25 in both HeLa cells and the normal human fibroblast cells, AG1521. Several antibodies for each protein were tested, and protocols are being refined to give the clearest results. We showed that the amounts of cyclin B1 protein are much higher in x-ray-exposed cells than in control cultures by three hours after x-irradiation. Surprisingly, there greater amounts seen in the nucleus as well as the cytoplasm of each irradiated cell. We are verifying this finding by fractionization of cellular components followed by western blot analysis.

In order to irradiate with alpha particles, the cells must be grown on mylar bottom tissue culture plates instead of glass slides. We have now optimized a method of handling the mylar-bound cells after

irradiation so as to be able to carry out the subsequent immunostaining steps in the visualization procedure.

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 make up one or more components of the multistep 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.

Publications, Presentations, and Other Accomplishments:

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Experimental Study of Nuclear Interactions Relevant to High Energy Heavy Ion Transport

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Funding:

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Solicitation:
Initial Funding Date: 10/94
Expiration: 12/96
FY 1995 Funding: $420,000
Students Funded Under Research: 6

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; 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) and possibly at other accelerators. The 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.

The first series of radiobiology and related physics experiments at the BNL AGS was completed successfully. Twelve radiobiology and four physics experiments were run. Our group had a large part of the responsibility for designing and setting up the radiobiology experimental facility at the AGS. We provided dosimetry and physics support for the radiobiology experiments. We carried out the physics experiments, which consisted of beam characterization and measurements of fragmentation in tissue and shielding material targets using solid state detectors, plastic nuclear track detectors and microdosimeters. Analysis of the physics data has begun. Fragment fluences for all targets have been extracted for Z=12-26, and fluences for Z=1-11 will be extracted during 1996. The feasibility of radiobiology with high energy heavy ions at the AGS has been established. Some technical details, such as how best to monitor beam uniformity, need to be addressed for the next AGS run in October 1996. Data from the fragmentation of high energy iron nuclei in tissue-equivalent and shielding targets must be compared to transport model predictions, so that the models can be refined and tested as necessary, perhaps with new shielding configurations. Data analysis continues on measurements of neutrons produced by heavy ions in thick targets. The final physics papers from high energy heavy ion running at the LBL Bevalac have been completed and are in press.

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.

Publications, Presentations, and Other Accomplishments:

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Zeitlin, C., L. Heilbronn, J. Miller, K. Frankel, W. Gong and W. Schimmerling "The fragmentation of 510 AMeV 56Fe in polyethelene." Radiat. Res., (in press).

Zeitlin, C., L. Heilbronn, J. Miller, W. Schimmerling, L.W. Townsend and J.W.Wilson "The fragmentation of 510 AMeV Iron in polyethylene: comparison between data and a Monte Carlo model." Sixth Annual NASA Space Radiation Health Investigators' Workshop, Brookhaven National Laboratory, Upton, NY, May 2-5, 1995.

Zeitlin, C., L. Heilbronn, J. Miller, W. Schimmerling, L.W. Townsend and J.W.Wilson "The fragmentation of 510 AMeV 56Fe in polyethylene: a comparison between data and a model calculation." Radiat. Res., (in press).

3D ORAM Dosimeter for Space Radiation Environments

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No Co-I's Assigned to this Task

Funding:

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FY 1995 Funding: $119,934

Students Funded Under Research: 2

Joint 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 mm^3) 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.

During the first year of the project, we developed a model to calculate the HCP induced "bit-flip" probability in single particle tracks. The model is based on: (i) the HCP track-structure described by the radial dose distribution, (ii) the spatial and temporal distribution of temperature in the HCP track, (iii) the matrix-specific radiation-induced changes, and (iv) the kinetics of transition of photochromic molecules from exited to ground state, following (ii) and (iii). To calculate the HCP track structure, we developed an analytical formula for the radial dose distribution D(r) in the ORAM material. The following assumptions were made: (i) only delta-rays are considered, (ii) atomic electrons are considered to be unbound and at rest, and (iii) delta-rays are assumed to travel in straight paths of length equal to their ranges. We do not expect that the above simplifying assumptions will have a significant effect on the outcome of the calculations. The energy deposited by the HCP is eventually dissipated as heat. We developed the formalism to enable the calculation on this temperature distribution and the resulting "bit-flip" probabilities. The theory was applied to a variety of HCP interacting in the 3-D ORAM material, including protons, alpha particles and oxygen in the energy range of 1 - 10 MeV/amu. Our results clearly demonstrate that cylindrical volumes of several microns in length (radius of a few nanometer) of radiation-induced bit-flips are formed. Furthermore, the shape and size of the volume affected by the radiation was shown to be dependent on the LET. Our theoretical results provide a strong indication that this method can become the basis for a crew dosimeter capable of LET discrimination. During the second year of the project we intend to continue according to the original

plan outlined in our proposal. Computer simulation will be used to describe how protons and other HCP are transported through the ORAM material. The methods that were developed during the first year of the project (the present year) will be used to simulate the bit-flip probability distribution within the particle tracks.

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, heaving 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.

Publications, Presentations, and Other Accomplishments:

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Patent Pending, U. S. Patent #: Undetermined, Moscovitch M. "Neutron Spectrometer, Real-Time Dosimeter, and Methodology Using Three-Dimensional Optical Memory, (Allowed: September 13, 1995)."

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No Co-I's Assigned to this Task

Funding:

Project Identification: 199-45-14-11

Solicitation:

Initial Funding Date: 10/94

Expiration: 12/97

FY 1995 Funding: $300,000

Students Funded Under Research: 0

Task Description:

No additional data was provided by the investigator for this research.

Effects of Exposure to Heavy Particles

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Funding:

Project Identification: 199-45-17-16
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1995 Funding: $26,782
Students Funded Under Research: 0

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 propose 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 1 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 1 GeV/n iron particles, measured using the conditioned taste aversion paradigm. These experiments were designed allow a comparison with the results obtained previously using 600 MeV/n iron particles and will establish a baseline for evaluating the results of subsequent experiments.

Continuing experiments are designed to determine the cellular mechanisms that mediate the neurochemical and behavioral changes produced by exposure to low doses of 1 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 Alternating Gradient Synchrotron (AGS) at Brookhaven National Laboratory (BNL) was available for a single set of experiments during October 1995. The goal of the experiments was to compare the effects of exposure to 1 GeV/n ^{56}Fe to the effects that had been previously obtained following exposure to 600 MeV/n ^{56}Fe using the BEVALAC at Lawrence Berkeley Laboratory (LBL). The specific endpoints were: 1) the behavioral toxicity of 1 GeV/n iron particles, measured using the conditioned taste aversion paradigm, 2) dopamine-mediated motor behavior, measured by studying upper body strength using a wire suspension task, and 3) oxotremorine enhanced dopamine release from striatal tissue, using HPLC. The doses tested ranged between 0.10 and 1.00 Gy. These doses were selected to bracket the range of effective doses for these endpoints determined for the 600 MeV/n ^{56}Fe exposures at

LBL. It was anticipated that these experiments would provide the baseline for future experiments using the AGS at BNL.

Exposing rats to 1 GeV/n ^{56}Fe particles produced a dose-dependent conditioned taste aversion; as the dose was increased, there was a corresponding increase in the intensity of aversion. Thus, as previously established for other types of radiation and heavy particles, the behavioral toxicity of 1 GeV/n ^{56}Fe is dose dependent. However, the behavioral toxicity of these particles was significantly less than that of 600 MeV/n ^{56}Fe particles. There was a 50% reduction in test day sucrose intake in rats exposed to approximately 0.20 Gy of 600 MeV/n 56Fe particles. In contrast, a dose of 0.50 Gy was needed to produce a 50% reduction in sucrose intake in rats exposed to 1 GeV/n ^{56}Fe particles. These results indicate that the behavioral toxicity of ^{56}Fe is dependent upon the energy of the particle. Because the LET of 1 GeV/n ^{56}Fe is ≈150 keV/μm, whereas the LET of 600 MeV/n ^{56}Fe ≈189 keV/μm, these results indicate that behavioral toxicity is partially dependent upon particle LET.

The second series of experiments examined signal transduction and dopamine release following exposure to 1 GeV/n ^{56}Fe particles. Statistical analyses indicated that there was a significant effect of irradiation on peak dopamine release following exposure to 0.50 Gy and that the effect of exposure to 0.10 Gy approached significance. These results are similar to those seen following exposure to 600 MeV/n ^{56}Fe, but there are differences in the specific pattern of the heavy particle-induced decrease in dopamine release. Thus, exposing rats to 1.0 Gy of 1 GeV/n ^{56}Fe particles did not produce a significant reduction in peak dopamine release. In contrast, exposure to both 1.0 or 0.50 Gy of 600 MeV/n ^{56}Fe particles did produce significant decreases in dopamine release when measured three days following irradiation.

Preliminary analysis of the dopamine-mediated motor behavior (wire suspension task) indicates that exposure to 1 GeV/n ^{56}Fe particles also produced a corresponding loss of upper body strength. However, as noted with the effects of irradiation on behavioral toxicity, exposing rats to 1.0 Gy of 1 GeV/n ^{56}Fe particles did not apparently produce a maximal loss of upper body strength.

The results of these experiments confirm that exposure to 1 GeV/n ^{56}Fe particles produces behavioral and neurochemical effects which are similar to those produced by exposure to 600 MeV/n ^{56}Fe particles. However, the relative effectiveness of these particles in producing behavioral and neurochemical changes is a function of the energy of the ^{56}Fe particles and, therefore, of particle LET (1 GeV/n ^{56}Fe ≈ 150 keV/μm; 600 MeV/n ^{56}Fe ≈189 keV/μm). Despite the fact that these initial experiments used doses that we anticipated would encompass the range needed to establish the thresholds and produce the maximal neurochemical and behavioral effects, this was not achieved. Therefore, during the next AGS run, several additional groups of rats will be run using higher dose exposures in order to achieve the maximal effects and to establish accurate ED50s for these effects.

Nonetheless, because the results of this run using 1 GeV/n ^{56}Fe particles at BNL did generally confirm our previous results, we can also move forward with research designed to expand the range of dopamine-mediated behaviors with are affected by exposure to ^{56}Fe particles and to establish the cellular mechanisms mediating the behavioral and neurochemical changes produced by exposure to these particles as detailed in the original proposal, including: 1) effects of exposure to ^{56}Fe on membrane fluidity and viscosity, 2) role of second messenger systems (e.g., inositol 1,4,5 triphosphate) mediating heavy particle-induces changes in signal transduction, and 3) effects of exposure on dopamine-mediated taste aversion learning.

The research which we have conducted previously has shown that exposing young rats (≈ 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 1 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 no 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.

Cooperative Radiation Research (NCI)

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Funding:

Project Identification: 199-08-17-64

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Students Funded Under Research: 0

Task Description:

This task is a collaboration between the NASA Office of Life and Microgravity Sciences and Applications (OLMSA) and the National Cancer Institute (NCI). The major goals of this collaboration are: 1) to enhance basic knowledge of living systems and their response to radiation exposure, 2) application of this knowledge to radiation protection, risk assessment, diagnosis, and treatment of cancer, and 3) exchange of technology applicable to problems common to OLMSA and NCI. Several researchers are associated with this joint effort:

Dr. David Chen (University of California) Radiation-induced Mutation Spectra at the HPRT Locus: The goal of the proposed work is to determine whether the phenomenon known as delayed mutation occurs in human cells exposed, *in vitro*, to ionizing radiation.

Dr. Eric Hall (Columbia University) The Effects of Small Doses of Radiation: Basic studies of the effects of neutrons and alpha particles on DNA-damage/repair, mutagenesis and transformation in mammalian cells at low doses and low dose rates of exposure.

Dr. Abraham Hsie (University of Texas Medical Branch) Molecular Analyses of Mammalian Radiation Mutagenesis: Molecular genetic study of the range of mutations and DNA damage induced by neutrons, x rays and alpha particles in human and rodent cell lines.

Dr. Amy Kronenberg (Lawrence Berkeley National Laboratory) Delayed Mutation and Instability in Human Cells: Molecular genetic study of the delayed mutagenesis and cell killing in human cell lines exposed to high-LET radiations.

Dr. Mitchell Turker (University of Kentucky) The Spectrum of Mutations Induced by Ionizing Radiations: A comparison of molecular mutation spectra induced in mammalian cells, including human cells, after exposures to gamma rays and alpha particles.

Dr. Charles Waldren (Colorado State University) Molecular and Cytogenetic Analysis of Mutation Induction: Analyses of small-scale point mutations and large-scale deletions on human chromosome 11 induced by low-dose x rays and neutrons at varying doses and dose rates.

Dr. David Chen:

Molecular analysis of γ-rays and α-particle induced HPRT mutants: There is preliminary characterization of 104 γ-ray induced, 121 α-induced and 25 spontaneously raised HPRT deficient human mutants. Both Southern and multiplex PCR methods have been used for the analysis. The structural alteration of the HPRT gene of the mutant DNA was compared to the control DNA pattern. Our results indicate that 44% of all γ-ray induced mutants have a normally structured HPRT gene and the remaining mutants (~56%) contain either partial deletion or total deletion of the HPRT gene. For a particle-induced mutant, ~37% of the mutants display a normal Southern/PCR pattern, whereas, ~63% show total gene deletion and partial deletion of the target gene.

Analysis of radiation-induced splicing mutations: We have modified a PCR procedure to synthesize HPRT cDNA from cell lysate of 1000 cells by reverse transcriptase. The synthesized cDNA is subsequently amplified by PCR using HPRT specific primers. All 6TGr deficient mutants with a normal HPRT structural gene have been used for the HPRT cDNA synthesis. These cDNA will be used for the SSCP analysis to identify point mutations. During the experiment, we have noticed that a number of mutants generated smaller HPRT cDNA.

Dr. Eric Hall

Project II: Normal human fibroblasts in plateau phase (G0/G1) were exposed to graded doses of carbon (C), oxygen (O), magnesium (Mg) and iron (Fe) particles (linear energy ranges from ~ 100 to 800 KeV/micrometer) generated by the accelerators at Brookhaven National Laboratory. Following irradiation, induced chromosomal changes were evaluated at the first mitotic division(all ions) and in the case of Fe ions at every passage to 4 weeks post irradiation. Charged particles appear to be efficient inducers of asymmetric chromosomal changes in the LET range ~ 100 to 800 keV/micrometer and in these preliminary studies showed no indication of induced genomic instability in these normal human cells. These results are different from reported induction of genomic instability in human fibroblasts exposed to iron and other ions and suggest possible differences in exposure conditions or LET may be important in the acquisition or non-acquisition of genomic instability in human cells exposed to HZE particles.

Project III - Transformation and Mutagenesis: Using a human papilloma virus immortalized human bronchial epithelial cell line it was demonstrated that heavy iron particles of ca 1 GeV/nucleon induced a dose-dependent toxicity with a mean-lethal dose of ~70 cGy. Compared to the 20 cGy dose value obtained with 150 keV/micrometer helium-4 ions, the high-energy iron particles were less effective in killing immortalized human epithelial cells under comparable culture conditions

Scientific Core and Project II: Experiments have been completed showing a very high cataractogenic potential for very low doses (2 to 250 mGy) of neutrons where very high RBEs are reported for other biological endpoints. Second, an experimentally-based "fingerprint" has been developed and verified for Hiroshima survivors to suggest a significant neutron component at Hiroshima. The approach opens the possibility of deriving human-based high-LET RBEs.

Dr. Abraham Hsie

The mutation spectra for alpha particles and x-rays in radiation-sensitive Chinese hamster ovary xrs-5 cells were not significantly different, but each was significantly different from the spontaneous mutation spectrum ($p<0.05$). As expected, deletions were the predominant mutational lesion for the ionizing radiations (ca 80%). Although not statistically significant, there was a suggestion from the data that deletions induced by exposure of cells to alpha particles were larger than those from gamma

radiation and may have been non-random. Analysis of larger numbers of HPRT mutants may reveal a signature different in the mutation spectra for these two forms of ionizing radiation.

Dr. Amy Kronenberg

Progress has been made toward the development of a series of syngeneic model cell lines which ectopically express the apoptosis control protein (bcl-2 or bclXL). Cells over-expressing each of these proteins have been established in syngeneic human lymphoblast differing in expression of the p53 tumor suppressor gene. Initial experiments showed that suppression of apoptosis results in enhanced survival in p53+ but not p53- cells exposed to high-energy (1 GeV/micrometer) iron ions. Experiments are continuing to determine the effect of suppression of apoptosis on mutation induction by HZE-accelerated charged particles.

Dr. Mitchell Turker

Mouse H22 embryonal carcinoma cells were used to measure the frequency and spectrum of mutations induced by alpha particles and gamma radiation. Mutations induced by the chemical alkylating agent EMS and by UV radiation were also used for comparison with the ionizing radiations. Partial analysis of mutations at the molecular level suggest that approximately 30% of the mutations induced by ionizing radiations were large mega-base-length deletions involving most of the selectable genetic marker (apart gene) as compared with less than 10% large deletions for spontaneous and much less than 10% large deletions for UV or EMS. At this fairly rough level of resolution there does appear to be different mutational signatures between ionizing these two ionizing radiations and chemicals or UV; however, it was not yet clear whether the mutation spectra of high-LET is different than that for low-LET radiation. Parallel *in vivo* experiments are being carried out to determine if the same mutational bias towards large deletions for high-LET also occurs in the whole animal.

Dr. Charles Waldren

The main goal of this work has been to quantify the number and the types of mutants that are generated in cultured mammalian cells exposed to various high- and low-LET radiations. Dose-response curves obtained with a hamster/human hybrid cell line with a selectable human chromosome (chromosome 11) showed that all of the high-LET radiations used (iron particles, nitrogen and helium-4) were more lethal and mutagenic than low-LET gamma rays. All forms of ionizing radiation in this study gave rise primarily to deletion mutations with the highest RBEs for iron particles (190 keV/micrometer) and nitrogen (126 keV/micrometer) (4 to 5) and lower values for helium-4 (150 keV/micrometers) (ca 2). Molecular-cytogenetic analysis of several hundred radiation-induced mutants showed that the mutational lesions for iron and nitrogen evolved over time after exposure, showing increasingly complex patterns of deletions, rearrangements and point mutations (i.e., indications of genomic instability). Conversely, low-LET-induced mutations appeared soon after exposure and were stable over time. This work suggests that both quantitative and qualitative differences exist between HZE particles and low-LET forms of radiation.

There are approximately 1 million new cancer cases every year, of which approximately one-half receive radiotherapy either alone or in combination with other treatment. Major advances in diagnosis and treatment have been made in recent years; they include imaging and treatment planning technology and the use of charged particle beams for radiation therapy. The cost of cancer to the nation has been estimated by the American Cancer Society in 1992 to be of the order of 100 billion dollars, of which half are medical expenses. Improvement in the detection and the treatment of cancer can thus be expected to significantly reduce human suffering and to have a large economic impact. Similarly, the uncertainties in prediction of radiation risks due to space radiation have been estimated to result in shielding requirements that may add tens of billions of dollars to the cost of a single mission beyond the Earth magnetic field. Improvements in the understanding of the biological action of space radiation are necessary to enable NASA to discharge its obligation to ensure the health, safety, and performance of astronauts at a significantly reduced cost.

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High-resolution Digital Mammography/NCI

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Solicitation:

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FY 1995 Funding: $250,000

Students Funded Under Research: 5

Joint Participation: NIH

Task Description:

The specific aims of this proposal are focused on the development, optimization and quantitative evaluation of a new digital mammography system with the following characteristics: 1) ≥ 90% quantum absorption efficiency, 2) 15 lp/mm limiting spatial resolution, 3) ≥ 0.5% contrast sensitivity, 4) ≥ 80% detective quantum efficiency, 5) 10^4:1 linear dynamic range (≥ 12) bits contrast resolution. The clinical aim of this proposal is to provide substantially improved diagnostic sensitivity for general screening.

This new approach is based on: 1) R&D performed by Nanoptics, Inc. for the Superconducting Super Collider, and 2) front-illuminated, thin-gate CCD technology developed by the Jet Propulsion Laboratory. It represents an excellent case of the union of NASA patented technology with technology supported by the Department of Energy. Nanoptics, Inc. has entered into a Strategic Alliance with Fischer Imaging to realize the goal of producing three prototype mammography units for installation at hospitals to conduct clinical studies.

We propose to develop a digital mammography system based on a scanning slot detector, which utilizes a novel plastic scintillating microfiber x-ray converter plate (1 cm x 20 cm x 1 cm thickness). A plastic scintillating fiber plate, whose microfiber axes aliened parallel to the direction of the incident x-ray provides a superior alternative to phosphor. We have recently achieved a technological breakthrough in doubling the energy conversion efficiency of our plastic scintillators. In order to increase the x-ray absorption efficiency, we have successfully incorporated 10% by weight of tin into the core of the scintillating microfibers. We have also succeeded in using very low refractive index cladding on our microfiber core to increase the tight output. Each individual microfiber will be parallax corrected by a thermoforming process to preserve the inherent high spatial resolution. This process has been successfully developed.

The microfibers within which the scintillating light is produced also directly transmit the light to the CCDs. The CCDs are protected from exposure to the x-ray beam by appropriate bends in the 7cm long continuous microfiber image guide. The CCD has appproximately 50% quantum efficiency at blue green region due to the thin-gate technology and has extremely low noise with cooling. The CCDs will

be operated in a time-delayed integration mode (TDI) which reduces the x-ray tube loading to an acceptable level and substantially eliminates artifacts in the image. The scanning system also facilitates the acquisition and handling of the large volume of data (155 Mbytes) associated with the high resolution image required for the given image size (8" x 12").

The first objective of the proposed research is to construct a section of slot detector connected to one CCD. This module will be tested extensively to determine all aspects of its performance parameters. Then, we will build one complete prototype slot detector covered by 4 CCDs including parallel readout of the CCDs. A complete system will be provided to Fischer Imaging for release as a mammography unit to the Moffitt Cancer Center, University of South Florida, to conduct clinical studies and evaluations for further improvement.

To summarize our progress after one year of work, we have:

1) successfully developed one plastic scintillating fiber slot module (SFP + bended image guide) with 7.5% tin loaded into the scintillating fiber core material. Using fluorinated polymer, we increased the scintillation light collection efficiency from typically 3% to 7.5%. The fiber core material also consists of a special developed scintillating dye (Nanoptics, Inc.) which has a measured energy conversion efficiency of 4.5% from x-ray photon energy to visible photon energy, a 1.5 times improvement compared to most plastic scintillators. An experiment to measure the scintillation light output from the SFP is being conducted.

These achievements are critical in realizing the goals of this research. The amount of scintillation light output from SFP determines, to a large extent, the ultimate detector DQE. From the above measurement, we estimate that the detector zero spatial frequency DQE is greater than 70% for typical detector exposure level (> 3 mR) encountered in mammography.

2) set up the testing system to perform the imaging performance measurements at Nanoptics, Inc. This setup includes a mammography x-ray unit (Senograph 500t), a modified PC-based high speed PCI bus frame grabber, and a linear scanning table with a computer-controlled motion controller which generates the synchronization signals for CCD camera electronics; the synchronization between object motion and CCD charge shifting is very important to achieve the goal of 15 detector-limiting spatial resolution. Also, it is critical to align the CCD columns to the scanning direction. In the proposed imaging system, the fast-scanning application requires the large amount of digitized image data to be acquired and stored in very short time. The success in setting up these components allows the prototype SFP detector imaging performance to be evaluated accurately.

3) built the CCD camera readout electronics. A circuitry for CCD dark current and detector non-uniformity corrections has been designed and is being integrated into the CCD readout electronics. A measured total thermal and readout noise level of 75 e⁻rms has been achieved at 2 MHz readout rate and 28°C. The CCD camera and readout electronics are being optimized for lower noise performance at present. This is another critical component in the prototype scanning slot digital mammography system which determines the detector DQE and the system linear dynamic range. Our goal is to obtain a total thermal and readout noise level of ~50 e⁻rms at 2 MHz readout rate and 25°C.

4) studied the effect of scattered radiation using Monte Carlo methods. It is found that the amount of scattered radiation present in the prototype imaging system is significant. An airgap method is found to be adequate to remove the scattered radiation. This technique will be employed in the digital mammography system being developed.

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 in missed in screening women using the existing Barographic 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 battle field patients.

There are major applications of large area, high resolution, real time digital radiographic cameras for industrial, aeronautical, and space applications. For example, high performance, composite materials are increasingly being used in systems 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.

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Space Radiation Transport and Interaction

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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 ionizing radiation than prior space 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 present in local tissues by adjusting the interaction with surrounding materials through materials 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 through materials including 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 as low as reasonably achievable (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, more recently, 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 illustrate 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/Continuous Electron Accelerator Facility, CEBAF), re-evaluation of the fragmentation database using the recent experiments of the BNL/AGS 1 AGeV Fe beam performed by J. Miller of LBL, development of higher order neutron propagators, determination of effects of current biophysical models on shield characterization, examination of dose rate effects in solar flare exposures (collaboration with Oak Ridge National Lab.), examination of effects of G1 and G2 blocking kinetics on cellular repair kinetics (collaboration with Johns Hopkins Cancer Research Institute), and development of analysis programs for space flight validation of environmental models, transport codes, and anatomical models (collaboration with JSC).

A high charge and energy (HZE) ion transport code has been written for use with the highly continuous space radiation boundary conditions with couplings to the light ion and neutron fields with the following physical assumptions: HZE ion fragmentation data base which includes projectile breakup assuming all fragment constituents have the same velocity as the ion before collision, no mesonic components, no gamma ray component, target fragmentation constituents only from the light ion and neutron collisions, no mesonic or gamma ray component for light ion or neutron collisions, only first order corrections in the regeneration source terms, straight ahead assumption, continuous slowing down approximation for ionic components, and neglect of the electromagnetic cascades from mesonic components. Although the code is regarded by many as best available (e.g., used for shuttle dosimetry, space station design, SEI studies, design of LIFESAT, chosen by Naval Research Lab. for Space Environment and Effects Project, high altitude aircraft studies, SAGE instrument design, etc.) further development is required. The code was originally written on the NUCFRG1 fragmentation database, augmented by a light ion database developed by Cucinotta (LaRC). Major modifications of the excitation energy distributions have occurred as a result of analysis of the 600 AMeV Fe experiments performed at LBL by J. Miller's group. The latest version of the HZETRN code uses the updated NUCFRG2 code as the nuclear fragmentation database. The analysis of the laboratory data required a newer version of transport code capable of meeting the near discontinuous boundary conditions associated with laboratory ion beams. This series of code uses the Green's function method and is referred to as the GRNTRN code. The GRNTRN code was further used to analyze data on C, N, and O ion beam experiments at the GSI facility. Added corrections have been added to the NUCFRG database to account for the effects of nuclear structure in the de-excitation process and the direct knockout of alpha clusters from the projectile nucleus. The important role of nuclear clusters was first observed by comparisons with JSC measurements with charged particle detector telescope on the space shuttle. The GSI comparisons further support the need for nuclear cluster knockout processes to be included in the fragmentation models. Indeed, the nuclear cluster models give the lowest lying nuclear excited states in the fragmentation process, and the few nucleon removal cross sections from all nuclei will not be adequately represented without a clearer description of the outer shell clusters of the normal nuclear state. Although an Optical Model Version of nuclear fragmentation (OPTFRG) had been developed utilizing Monte Carlo statistical description of the nuclear state even prior to the first NUCFRG code, this code never developed into a database generator due to the excessive computer requirements. More recently a Quantum Multiple Scattering fragmentation code (QMSFRG) has been developed which is capable of including nuclear cluster knockout, evaluates the knockout spectra, evaluates the excitation spectra of the nuclear fragmentation, and conserves energy in the reaction. All of these features were missing in the OPTFRG and the NUCFRG models. A new version of nuclear de-excitation based on master equations has shown great promise in evaluation the final fragment distribution as a result of

the simplifying form of the emission spectrum at high excitation energies. An alliance to develop nuclear cluster wave functions to further promote the QMSFRG model development for the vast array of ions in the cosmic environment has started in collaboration with researchers at the new DoE/Continuous Electron Beam Accelerator Facility through Hampton University. Understanding nuclear clusters is part of the charter of the CEBAF mission. Biophysical modeling has been improved through a re-evaluation of the secondary electron distributions and development of survival/mutation competition models for analysis of shield worth. Dose equivalent appears to be a misleading guide to shield worth in most materials.

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 (SEU) in modern aircraft designs and spacecraft designs.

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Energetic Proton Dose-Response

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FY 1995 Funding: $55,400

Joint Participation: DoD

Solicitation:

Expiration: 3/95

Students Funded Under Research: 0

Task Description:

This project was begun with support of a previous NASA Life Sciences grant and continues under the present grant. It is a life span dose response study of tumor risk after exposure of the head to energetic protons. Eleven hundred male Fischer-344 rats, aged 70 days, were divided into five dose groups of 200 animals each, with an additional 100 animals retained for quality control monitoring. The dose groups were zero (sham), 2, 4, 8.5 and 18 Gy. At 923 days after irradiation, with less than 2% of the subjects alive, all remaining animals were sacrificed and examined. Every subject in the study received a complete post-mortem examination, including serial sections of the brain for histological verification of tumor occurrence and type. Total head and neck tumor incidence in the dose range of 0-8.5 Gy revealed a linear dose-response. The exposed rats had a greater incidence of tumors, especially pituitary chromophobe adenomas, epithelial and mesenchymal cell tumors, than the unexposed controls, but the excessive occurrence of malignant gliomas that had previously been observed in proton-irradiated monkeys was absent in the rats. The estimated dose required to double the normal population incidence of all types of head and neck tumors was 2.7 Gy. The highest dose, 18 Gy, resulted in high mortality due to obstructive squamous metaplasia of the upper respiratory tract during the first 12 months after irradiation. In a subsequent study, rats were exposed to g radiation on the same dose and dose rate schedule as the proton-exposed rats in order to establish the relative biological effectiveness of the proton radiation in producing the observed lesions. Five groups of 40 animals were exposed while restrained in plastic rat holding cylinders shielded by lead so that only the head received radiation. They were observed for the remainder of their life span, which was completed in January, 1995. All animals received a complete post-mortem examination, including multiple sections of the brain for detection of microscopic lesions.

The in-life portion of this study was completed in January, 1995, with tabulation of the mortality data and preservation of the tissues from all test subjects. Work for the second grant period consists of processing the preserved tissues, classifying the tumors and making statistical comparisons of the

tumor incidence in the proton-exposed and 60Co g-radiation-exposed animals. All work including the final report is expected to be completed by 30 September, 1996.

An understanding of the cancer risk from space radiation is necessary for the establishment of radiation safety guidelines and procedures for manned missions in space. The potential benefits to earth bound populations are more realistic assessments of cancer risks from environmental radiation sources and improved radiation protection procedures.

Publications, Presentations, and Other Accomplishments:

D.H. Wood, A.B. Cox, K.A. Hardy, Y.L. Salmon and R.W. Trotter "Head and neck tumors after energetic proton irradiation in rats." Adv. Space Res., vol 14, no 10, 681-684 (1994).

Neoplastic Cell Transformation With Protons and HZE

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Responsible NASA Center: Johnson Space Center

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 3 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.

These research studies are specifically designed to test the hypotheses that galactic cosmic radiation increases cell inactivation and neoplastic transformation in mammalian cells and that heavy ions with energies greater than 1 GeV/u can have high RBE and can effectively produce irreparable oncogenic lesions. The primary goal of this project is to obtain quantitative information on how mammalian

cells respond to energetic protons and high-energy heavy ions. A crucial secondary goal is to determine the repair of radiation-induced damage and the oncogenic effects of gamma rays at very low dose rate. The specific aims are as follows: 1) To investigate the lethal and oncogenic effect of fragmentation of tissue-equivalent materials caused by protons, 2) to determine the RBE of selected heavy ions having energies greater than 1 GeV/u, 3) to examine the kinetics of repair of proton- and heavy-ion-induced lethal and oncogenic damage in mammalian cells, 4) to study the oncogenic effect of gamma rays at very low dose rate (<0.05 cGy/min) in confluent cells, and 5) to clone and characterize radiation-transformed human epithelial cells.

During this reporting period, we conducted experiments with 250 MeV protons at Loma Linda University Medical Center (LLUMC), with 1 GeV/u iron beam at Brookhaven National Laboratory, and with 290 MeV/u carbon particles at the National Institute for Radiological Sciences (NIRS), Chiba, Japan. In addition, we performed cell fusion experiments to determine whether the transforming gene(s) is dominant or recessive. Furthermore, we carried out a systematic investigation to determine possible alterations in chromosomes and cancer genes in human mammary epithelial cells transformed by heavy ions.

An experiment to determine the cellular effects of 250 MeV protons at various depths in tissue has been performed. Mammalian cells at Go/G1 were shielded with various thickness polyethylene and exposed to 250 MeV protons generated by the accelerator at LLUMC. Dr. Dan Miller, the Chief Clinical Physicist of Department of Radiation Medicine at LLUMC, performed the dosimetry, and Dr. Daila Gridley of Department of Microbiology at LLUMC provided assistance in using tissue culture facilities. Preliminary results indicated that the survival curves were similar for 250 MeV protons with residual range 31.5-, 16-, and 0.3-cm in tissue. When cells were held at confluent state for 1-day at 37°C after irradiation, there was a significant increase of survival, even for protons with residual range 0.3-cm in tissue. The effectiveness of protons in causing aberrations of chromosome #2 and #4 in human diploid fibroblasts was also determined, using fluorescence *in situ* hybridization (FISH) technique. The dose-response curve was curvilinear and similar for protons with and without shielding.

Similar to the proton experiment, we irradiated both C3H10T1/2 cells and human diploid fibroblasts with 1 GeV/u iron particles accelerated at AGS of Brookhaven National Laboratory. Due to the limitation of beam time, we only studied the effects of 0- and 5-cm water shielding. Preliminary data indicated that the survival curves for 0- and 5-cm shielding were similar and close to exponential. Interestingly, the survival curves for immediate and delayed plating were similar, suggesting no repair of potential lethal damage. In addition to shielding and repair studies, we also performed a simple experiment with dimethyl sulfur oxide (DMSO) to determine the importance of indirect effect. Confluent cells were exposed to 2M DMSO shortly before and during irradiation and were plated either immediately following exposure or 1-day after irradiation. Results showed no effect of DMSO on survival, suggesting that the lethal damages were induced primarily by direct interaction between iron particle and nuclear DNA molecule.

Experiments with 290 MeV/u carbon ions were done recently and are in progress. Confluent mammalian cells were exposed to carbon beam with 0- and 5-cm polyethylene. Preliminary results will be obtained within next two months. For determining the oncogenic effects of ionizing radiation in human epithelial cells, we transformed a mammary epithelial cell line immortalized by benzo(a)pyrene, with energetic heavy ions and obtained several transformed clones. These transformed cells showed similar growth properties on Matrigel as human mammary tumor cells. To better understand the mechanisms of radiogenic transformation of human cells, we systematically examined the alterations in chromosomes and cancer genes. More than 14 different chromosomes were examined for translocations, using FISH technique, and chromosome #3, 13, 14, 16 and 18 appeared to be normal in transformed cells. However, chromosome #1, 8 and 17 in transformed cells showed different pattern from those in nontransformed cells. Southern blot analyses indicated no detectable alterations in myc, ras, Rb and p53 genes. Further studies of chromosome #17 by *in situ* hybridization with the unique sequence p53 gene probe and a centromere probe showed no loss of p53 gene in transformed

cells. These findings were presented at a workshop on neoplastic transformation in human cell systems in culture, held in Chicago, Illinois on Sept. 1-9, 1995.

For a better understanding of basic mechanism(s) of radiogenic cell transformation, we completed a cell fusion experiment. This type experiment is essential to learn about the nature of cancer gene(s) in oncogenic transformation by ionizing radiation. The technique used to fuse non-transformed cells with transformants was DMSO-PEG method. Nontransformed cells were resistant to both 6-thioguanine and ouabain, and transformants were sensitive to both drugs.

The nontransformed cells with these double markers were selected in our laboratory after considerable effort. Large colonies were found within four weeks of incubation after cell fusion. Fused cells survived in the selection medium were cloned and analyzed for karyotype. The chromosome number varied from 80 to 92 with an average about 86 and a modal number 85, which is near tetraploidy.

These fused cells showed density inhibition of growth. No pilling-up of cell, i.e., foci, was observed in dishes with cells confluented for several weeks. These results suggest that the oncogenic property of human mammary epithelial cells transformed by ionizing radiation is recessive, and thus tumor suppressor gene(s) can be important in radiation carcinogenesis.

Based on the results obtained, we plan to conduct experiments with 250 MeV protons for oncogenic cell transformation determination. With the survival curves obtained, cell transformation experimentas can be designed properly. The transformation frequency is known to be cell density dependent. Without the information on survival, one may plate too many survivors and thus may suppress the formation of foci. We also plan to do experiments with 1 or 8 GeV/u iron particles accelerated at AGS of Brookhaven National Laboratory to determine RBEs, repair of potential lesions, and shielding effects. The molecular analysis of human mammary epithelial cells transformed by heavy ions will be continued. Southern blots of tumor suppressor genes other than Rb and p53 will be done, and the expression of cancer genes will be explored. If funding and beam time are available, we plan to perform fragmentation studies with neon or argon ions, in collaboration with scientists at NIRS in Chiba, Japan.

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.

Publications, Presentations, and Other Accomplishments:

George, K., and Yang, T.C. "Chromosomal translocations in human cells exposed to gamma rays." 43rd Annual Meeting of Radiat. Res. Soc., San Jose, CA, April 1-6, 1995 (Abstract).

Wu, H., Goodwin, E.H., and Yang, T.C. "Spatial consideration in the formation of radiation-induced chromosome aberrations and the test of interaction distance hypothesis." 43rd Annual Meeting of Radiat. Res. Soc., San Jose, CA, April 1-6, 1995 (Abstract).

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Appendix

REPORT DOCUMENTATION PAGE

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