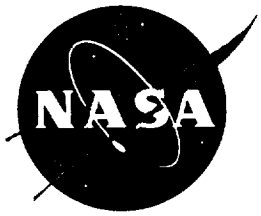
NASA Technical Memorandum 4801



Life Sciences
Program Tasks and Bibliography for FY 1996

National Aeronautics and Space Administration

Office of Life and Microgravity Sciences and Applications

Life Sciences Division

May 1997

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 1997	Technical Memorandum

4. TITLE AND SUBTITLE
Life Sciences Program Tasks and Bibliography for FY 1996

5. FUNDING NUMBERS
NASw-5000

6. AUTHOR(S)
Edited by John C. Nelson

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
National Aeronautics and Space Administration
Office of Life and Microgravity Sciences
Washington, DC 20546-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
National Aeronautics and Space Administration
Washington, DC 20546

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
NASA-TM-4801

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Unclassified - Unlimited
Subject Category - 51

12b. DISTRIBUTION CODE

13. ABSTRACT (maximum 200 words)

This document includes information on all peer reviewed projects funded by the Office of Life and Midrogravity Sciences and Applications, Life Sciences Division during fiscal year 1996. 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.

14. SUBJECT TERMS
Life sciences, bioastronautics, aerospace medicine, spaceborne experiments, biological effects, microgravity exobiology, life support systems, bibliographies

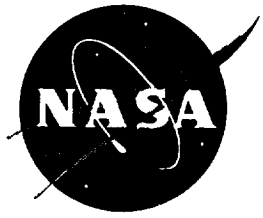
15. NUMBER OF PAGES
940

16. PRICE CODE
A99

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclass	Unclass	Unclass	Unlimited

Available from NASA Center for AeroSpace Information
800 Elkridge Landing Road
Linthicum Heights, MD 21090-2934
(301) 621-0390

NASA Technical Memorandum 4801



Life Sciences
Program Tasks and Bibliography for FY 1996

Office of Life and Microgravity Sciences and Applications

Life Sciences Division
Code UL

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

May 1997

I. Introduction

II. Life Sciences Program Tasks

Flight Mission / Programs

Bion

Biorack

Biospecimen Sharing

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Euro-Mir

Life and Microgravity Spacelab (LMS)

NASA-Mir-1B

Neurolab

Spacelab-Mir-1A (SLM-1A)

Space and Life Sciences-2 (SLS-2)

Small Payloads

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Environmental Health

Radiation Health

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Cellular and Molecular Biology

Developmental Biology

Education

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NASA Specialized Centers of Research and Training (NSCORT)

Appendix

I.
Introduction

TASKBOOK INTRODUCTION FOR FY 1996

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

The Office of Life and Microgravity Sciences and Applications (OLMSA) is responsible for planning and executing research stimulated by the Agency's broad scientific goals. OLMSA's Life Sciences Division is responsible for guiding and focusing a comprehensive program of flight and ground-based tasks. This document, the Life Sciences Program Tasks and Bibliography for Fiscal Year 1996 (October 1995-September 1996), includes all peer reviewed projects funded by the Office of Life and Microgravity Sciences and Applications, Life Sciences Division, during that year. This document is 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 (http//www.hq.nasa.gov/office/olmsa/UL/codeul.html). The information provided in the Task Book is used in reports to the NASA Associate Administrator, the Office of Management and Budget, and to the United States Congress.

In this book, flight tasks are organized by flight mission/program while ground-based tasks are divided into thirteen elements within four major scientific programs. The on-line version of this publication also offers the option of searching all tasks by scientific program, element, and/or discipline. A complete listing of scientific programs and elements, as well as flight missions/programs, is provided on pages I-4 and I-5.

It should also be noted that the FY 1996 funding amounts given in this publication for ground tasks represent funds appropriated from the fiscal year 1996 NASA budget, and do not represent funding allocated from other fiscal year budgets or other agencies. FY 1996 funding amounts for flight tasks were provided by the individual principal investigators.

The Life Sciences Division wishes to thank Information Dynamics, Inc., and Universities Space Research Association personnel at NASA Headquarters and in particular recognize John Nelson (task book review process and publication manager), Bob Dunning, Elaine Makovska, Jennie Moehlmann, Lynne Powell, Keith Robertory, Lori Tyahla, and Bill Wilcox for their efforts in the development, compilation, and publishing of this report. Gratitude is also expressed to the following people who were responsible for coordinating flight task data delivery from NASA field centers: Bonnie Dalton and Frances Acosta at ARC; Dr. Jerry Homick, Elisa Allen, Sharon Jackson, and Bonnie Meadows at JSC; Dave Reed, Doug Gruendel, and Ray Wheeler at KSC.

FY 1996 PROGRAM RESEARCH TASK SUMMARY: Overview Information and Statistics

Total Number of Principal Investigators: **283**

—

Total Number of Tasks: **364**

—

Total Number of Bibliographic Listings: **1711**

—

Number of Students Funded: **1302**

—

Number of States with Funded Research (including District of Columbia): **41**

—

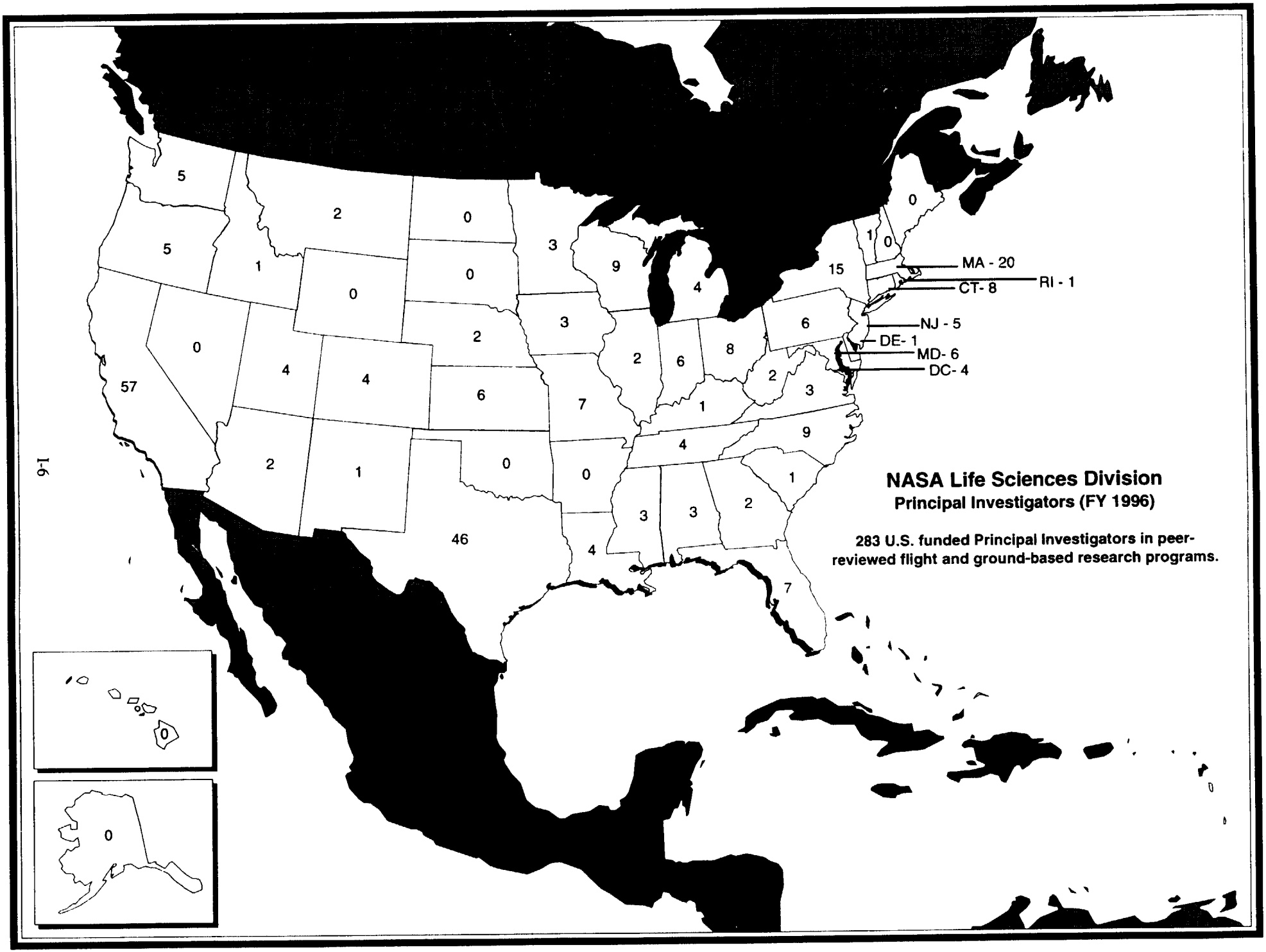
FY 1996 Life Sciences Budget: ..... **$109.6 Million**
(Ground-based research: $55.2 Million; Flight-based research: $54.4 Million)

Number of Tasks listed by Program and Element (210 Ground, 154 Flight):

Program	*Element*	*Ground Tasks*	*Flight Tasks*	*Total Tasks*
Advanced Human Support Technologies	Advanced Environmental Monitoring and Control	14	2	16
	Advanced Life Support	14	1	15
	Space Human Factors Engineering	8	0	8
	Total	**36**	**3**	**39**
Biomedical Research and Countermeasures	Behavior and Performance	7	7	14
	Environmental Health	7	6	13
	Radiation Health	20	4	24
	Space Physiology and Countermeasures	87	74	161
	Total	**121**	**91**	**212**
Gravitational Biology and Ecology	Cellular and Molecular Biology	17	14	31
	Developmental Biology	5	33	38
	Education	1	0	1
	Plant Biology	19	13	32
	Remote Sensing and Ecology	1	0	1
	Total	**43**	**60**	**103**
NSCORT		**10**	**0**	**10**

Number of Flight Tasks listed by Flight Mission/Program:

Flight Mission/Program	*Number of Tasks*
Bion	10
Biorack	8
Biospecimen Sharing	2
Cosmos 2229	1
Euro-Mir	10
LMS	13
NASA-Mir-1B	19
Neurolab	25
SLM-1A	25
SLS-2	1
Small Payloads	40



NASA Life Sciences Division
Principal Investigators (FY 1996)

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

II.
Life Sciences
Program Tasks for FY 1996

- **Flight Research**

- **Ground-based Research**

Adaptive Response of Slow and Fast Skeletal Muscle in the Monkey to Spaceflight

Principal Investigator:

Sue C. Bodine, Ph.D.
previously associated with the
University of California, San Diego - School of Medicine

Phone: 914-345-7755
Congressional District: -

Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 106-30-45

Initial Funding Date: 4/91

FY 1996 Funding: $45,700

Solicitation: NRA 88-OSSA-8

Expiration: 6/96

Students Funded Under Research:

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

Task Description:

No additional information was supplied by the principal investigator.

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

Principal Investigator:

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Congressional District: NY - 14

Co-Investigators:

Theodore Raphan, Ph.D.; CUNY, Brooklyn College
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: 9/88

Expiration: 12/96

FY 1996 Funding: $

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion

Responsible NASA Center: ARC

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. 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 GIA on the 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 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.

Our hypotheses include the following:
A. Otolith ocular reflexes induced through the lVOR, such as 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.

Acomplishments during FY96 include:

1. UPGRADE OF 3 AXIS ROTATOR: We upgraded the primate axis drive motor on our 4 axis COSMOS rotator so that it could provide smooth, controlled acceleration during velocity steps and could deliver sinusoidal linear acceleration. This work was done in anticipation of participating in the 1996 BION Mission. Subsequently, our Vestibular/Oculomotor Experiment was removed from the Manifest of the 1996 BION Mission. If there is a repeat BION flight in which we can record eye movements of monkeys before and after flight, the rotator will be ready for this contingency.

2. EVALUATION OF TECHNIQUE FOR IMPLANTING THREE-DIMENSIONAL EYE COILS: The failure rate of scleral search coils in the 1992-1993 COSMOS Mission was about 50%. We reevaluated our surgical procedures and the techniques for making eye coils. In five 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. Subsequently, seven additional operations have been done on monkeys utilizing both frontal plane and torsional eye coils. There was a zero rate of failure. It appears that the eye coil wires were over-tightened when they were wound to make twisted pairs, and that this accounted for the high failure rate in the COSMOS 2229 Mission. Regardless, the problem of long-term implantation has been solved satisfactorily for any subsequent usage.

3. BASELINE DATA COLLECTION FOR CENTRIFUGATION AND FOR VERGENCE ASSOCIATED WITH OVAR: We did baseline data collection in monkeys implanted with eye coils that record eye position in three dimensions. The purpose was 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. New software was written to collect and analyze six channels of eye position data (horizontal, vertical, and roll for both eyes). We also updated equipment to prepare for the flight. Specifically, eye coil recordings of horizontal, vertical and roll eye position were done for each eye. This permits analysis of the signals from both eyes during testing.

4. Analysis of COMOS 2229 data: We published the following results from the 1992-1993 COSMOS 2229 Mission:

A. 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 was the first time that it had been demonstrated that there is a clear and long lasting effect of space flight on otolith-ocular reflexes. (Dai, M., McGarvie, L., Kozlovskaya, I., Raphan, T. and Cohen, B. Effects of spaceflight on ocular counterrolling and the spatial orientation of the vestibular system. *Exp. Brain Res.* 102: 45-56, 1994).

B. The orientation axis of velocity storage changed from a gravitational reference before flight toward a body reference after flight (Ibid). This is consistent with our results in the COSMOS 2044 flight (Cohen, B., Kozlovskaya, I., Raphan, T., Solomon, D., Helwig, D., Cohen, N., Sirota, M., and Yakushin, S. The vestibulo-ocular reflex (VOR) of rhesus monkeys after spaceflight in the COSMOS biosatellite 2044. *J. Appl. Physiol.*, 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.

C. 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 (Dai et al. 1996). That is, because of gravity, the eyes converge when the head is up, and diverge when the head is down. 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. I t provides a new, robust, and relatively simple technique for testing otolith-ocular function at various levels of gravitational acceleration.

5. Vergence associated with linear acceleration during OVAR was greatly attenuated after the 2229 Mission for a prolonged period after flight (>11 days) (Dai et al. 1996). This finding has implications for visual function in space, since vergence during forward translational movements would be absent or attentuated in space.

6. NASA support was critical for holding a Conference on New Directions in Vestibular Research at the Rockefeller University in New York City in June 1995. A volume was published from this Conference in the Annals of the New York Academy of Sciences, (Volume 781).

The proposed research will determine how otolith-ocular reflexes are altered after adaptation to space. In particular, we will show how OCR, ocular vergence, and spatial orientation of the 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 1-G terrestrial environment of Earth. The information will also be used to direct countermeasures to overcome lags in adaptation or changes in gaze and balance due to the abnormal force field environment of microgravity. Such information and countermeasures will be critical 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 VOR which will include both angular and linear acceleration inputs, and which will account for dynamic changes that alter the orientation of the system vectors to those of gravito-inertial acceleration. 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 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.

FY96 Publications, Presentations, and Other Accomplishments:

Arai, M., Dai, M., Raphan, T., and Cohen, B. (abstract) Full-field optokinetic nystagmus induced in whole body tilt positions. Vestibular Res., 6:4S:, S18 (1996).

Cohen, B. (abstract) Spatial orientation of the angular vestibulo-ocular reflex (aVOR): velocity storage of monkeys and humans. Vestibular Res., 6:4S, S67 (1996).

Cohen, B. (abstract) The functional significance of the spatial orientation of optokinetic nystagmus and centrifugation. Vestibular Res., 6:4S, S51 (1996).

Cohen, B. Abstract. 19th Meeting of the Barany Society, Sydney, Australia, August 11-14, 1996.

Dai, M., Raphan, T., Kozlovskaya, I., and Cohen, B. "Vestibular Adaptation to Space in Monkeys" in "Otolaryngology, Head and Neck Surgery." Edited by: Honrubia, V. (in press).

Dai, M., Raphan, T., Kozlovskaya, I., and Cohen, B. (abstract) Modulation of ocular vergence by off-vertical yaw axis rotation in monkeys: normal characteristics and effects of space flight. Vestibular Res., 6:4S, S65 (1996).

Dai, M., Raphan, T., Kozlovskaya, I.B., and Cohen, B. Modulation of vergence by off-vertical yaw axis rotation in the monkey: Normal characteristics and effects of space flight. Exp. Brain Res., 111, 21-29 (1996).

Highstein, S.M., Cohen, B., and Büttner-Ennever, J.A. New directions in vestibular research." Annals of the New York Academy of Science, vol. 781, 1996.

Raphan, T. and Cohen, B. "How the VOR works: Spatial orientation of the vestibulo-ocular reflex in monkey and man" in "Handbook of Clinical Neuro-Otology." Edited by: Baloh, R.W. and Halmagyi, G.M. Oxford University Press, vol. 1, pp 20-47, 1996.

Functional Neuromuscular Adaptation to Spaceflight

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Dr. John Hodgson; University of California, Los Angeles

Funding:

Project Identification:
Initial Funding Date: 1/95
FY 1996 Funding: $

Solicitation: NRA 88-OSSA-8
Expiration: 12/95
Students Funded Under Research: 17

Flight Information:

Flight Assignment: Bion 11, 9/96
Responsible NASA Center: ARC

Task Description:

All of the data from Bion 11 has now been collected. Sensors for EMG and tendon force performed extremely well, providing useful data well beyond their specified life. All of the planned biopsies were taken and are currently being analyzed.

Treadmill locomotion tests have provided the first recordings of the force of an individual muscle in a walking monkey both before and after space flight. Preliminary comparisons with recordings after flight suggest that the soleus muscle EMG activity of both monkeys during locomotion is reduced after space flight, consistent with our findings of reduced soleus activity in the foot lever task after Bion 9 and 10. Review of flight and pre-flight capsule test data from both animals indicates high-quality recordings throughout the capsule testing.

The primary objective during this period was to participate in final joint bioengineering tests and in pre-flight activities in preparation for flight. This included collection of all pre-flight data, evaluation of sensor status immediately prior to flight, and participation in the selection of flight animals.

A total of 6 Rhesus monkeys were implanted with EMG electrodes and a tendon force transducers to facilitate final Joint Bioengineering Tests JBET) of the flight hardware and to provide pilot data to use during development and testing of data analysis procedures.

Twelve flight candidates were trained and implanted. Due to time constraints and some undesireable interactions between training protocols for the different tasks required of the flight monkeys, behavioral training and testing was limited to quadrupedal treadmill locomotion with some evaluation of natural postures adopted between walking sessions on the treadmill. Recordings were made at treadmill speeds of 1, 2, 3, and 4 MPH. All of the monkeys walked easily over this range of speeds, and no evidence of distress was observed when monkeys were

on the treadmill. Locomotion data were recorded via telemetry link onto an analog tape recorder and later digitized and analyzed by computer.

EMG and force transducer data were recorded continuously from the monkeys during several 24-hour periods of the normal daily routine. The telemetered data were recorded directly onto a digital computer. Software has been developed in LabVIEW to create hourly amplitude histograms which are then transferred to spreadsheet templates for graphing and calculation of integrated EMG activity.

Recordings were also made during the foot lever testing. These data have been evaluated but not analyzed. Signal quality appears to be excellent, and we envisage no problems with analysis of these pre-flight data.

The animals selected for flight had a full complement of sensors applicable to this project, and review of the flight data indicated that excellent quality data were recorded from all sensors in both flight animals. Pos-tflight testing of flight and control animals was completed in early 1997, and our task for the remainder of the year will be to complete analysis of the data collected and perform, some early activities such as additional bioengineering tests for BION 12.

Analysis of pre- and post-flight locomotion data shows that soleus EMG activity was reduced and medial gastrocnemius activity (force and EMG) increased following the 14-day space flight. Subsequent recordings in one animal provided similar observation for one week following the flight, after which EMG activity levels returned to pre-flight control levels. Similar observations but with much smaller changes in magnitude were made in control animals which were restrained for a 14-day period at 1-G.

These data strengthen our previous findings that there appears to be some reorganization of motor control in response to microgravity such that the relative activation of slow extensor muscles becomes lower when compared to fast extensors.

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 considerably clearer understanding of the physiological signals which may contribute to the maintenance of muscle mass. For example, the activity levels in muscles of the legs will be monitored during normal activities at normal gravitational loading as well as in the microgravity environment. These data should indicate the importance of activity in maintaining normal mass and functional properties of flexor and extensor muscles. The role of activity of specific muscles in maintaining normal levels of control of movement also will be determined. One of the major advantages of the proposed experiments in efforts to understand basic biological processes is that the normal neuromuscular system will be studied in an abnormal physiological environment, i.e., the altered function is caused by an altered environment, not an altered capability of the physiological system being studied as would be the case with surgical or pharmacological manipulation.

Each phase of these experiments has important implications on the optimization of rehabilitative care in addressing problems related to neuromuscular dysfunction as well as some aspects of hormonal function. These results could have a fundamental and large impact on currently 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.

Effect of Weightlessness on Single Muscle Fiber Function in Rhesus Monkeys

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Congressional District: WI - 5

Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 2/95

FY 1996 Funding: $120,000

Solicitation: NRA 88-OSSA-8

Expiration: 1/96

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

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 will be quick frozen in liquid nitrogen and the other placed in skinning solution (-20^0 C). The frozen samples will be freeze-dried and stored under vacuum (-80^0 C) for subsequent biochemical analysis, while the skinned fiber bundle will be used to study the physiological properties of individual fast- and slow-twitch fibers.

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

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

Following each of the physiological and biochemical studies described above, a section of the fiber will be loaded on a 5% SDS-PAGE gel to assess the myosin heavy 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 spent the majority of our time conducting a pre-flight analysis of the functional properties of individual muscle fibers isolated from the gastrocnemius and soleus muscles of the 12 flight candidates. After obtaining the biopsy, we divided the sample such that one part was quick frozen in liquid nitrogen and freeze-dried at -40°C, and the other was placed in cold (4°C) skinning solution. Following a 24-hr soak, the solution was exchanged for fresh skinning, and the sample was stored until used at -20 °C. The freeze-dried samples were stored under vacuum (-80°C). These samples will be used to isolate individual slow- and fast-twitch fibers for biochemical analysis. However, these substrate and enzyme analyses will not be carried out until post-flight so that both the pre- and post-flight samples can be studied under the exact assay conditions. The physiological studies of the pre-flight samples have been completed, and these results are described below.

Physiological Studies. On an experimental day, single fibers were isolated from either the soleus or gastrocnemius and suspended between an isometric force transducer and a position detector. After equilibration at 15°C in relaxing solution, the fiber was set to its optimal length, and a Polaroid picture was taken at 800X magnification while the fiber was briefly suspended in air. From this micrograph fiber, diameter was measured, and fiber cross-sectional area was calculated. For each fiber, we then determined peak force, maximal shortening velocity (determined by the slack test), force-velocity relationship, force power relationship, fiber stiffness, and the pCa- force relationship. As expected, the fast fiber types showed significantly higher maximal shortening velocities and peak powers compared to the slow-twitch fibers. Interestingly, the diameter of the slow type I fiber of the gastrocnemius was significantly smaller than the other fiber types. Our hypothesis is that this relative atrophy is a result of the monkeys spending prolonged periods of time in a squatting position. This posture would tend to unload the gastrocnemius and reduce the reflex activation of the slow fiber type. In the next contract year, similar studies will be conducted on the flight and growth control monkeys, and the results will be compared to these data to determine the effects of weightlessness on the functional properties of fast and slow limb skeletal muscle fibers. Following each experiment, fiber type was determined by 5% SDS gel analysis of the myosin heavy chain. 12% SDS gels were also run for each fiber so that changes in the myosin light chain and/or regulatory proteins could be determined.

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.

Homeostatic and Circadian Responses of Rhesus Monkeys During Space Flight

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Project Identification:

Solicitation: NRA 88-OSSA-8

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $130,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

Task Description:

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

The Bion 11 flight scheduled for mid-summer 1996 was slipped to late December. Pre-flight preparations were also moved to accommodate that schedule. These have included our participation in meetings and discussions concerning flight and ground-based experimentation, generation of supporting documents, and development of sensors.

During an Investigator Working Group 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 were held at Ames Research Center to serve the purpose of moving preparations forward for flight and ground-control studies, including equipment verification tests.

The Bion Program was again reviewed, this time by a "Blue Ribbon" Panel. We presented the Regulatory Team science objectives and integration plan to the Bion Program Review Panel.

We continued to actively participate in the writing and editing the Experiment Management Plan (EMP), including the three Regulatory Discipline EMPs (Thermoregulation, Circadian Rhythms, Metabolism and Sleep) and the Integrated EMP (IEMP) with our French and Russian colleagues. In addition, we developed and presented the Regulatory Team science objectives to the Bion Program Review Panel.

We have continued evaluation of the design and location of the deep body temperature sensor location and probe design. To date, these studies have been performed at Ames Research Center.

We completed our analysis of the melatonin, body temperature, and heart rate data from the Adult Rhesus Restraint Test conducted at Ames Research Center. 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. 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 may diminish in rhesus and humans. These pathologies may arise not only in environments without time cues, such as constant light (or constant dim light found in many of today's intensive care units), but also with shifts in time zones, shift work and in aging individuals where internal temporal coupling appears weakened. Narcolepsy is a class of diseases in which daytime sleep attacks or Rapid Eye Movement (REM) sleep onset can occur. Some of these individuals display a loss of circadian patterns of REM sleep distribution. Further, when the individuals are tested for sleep latencies throughout the 24-hour day, there is often lack of circadian variation in the sleep latency as compared with the normal subjects. Other instances have been studied in which individuals cannot synchronize themselves with their environment and maintain a 24-hour day, but rather free-run with a circadian 25-hour day. Phase relationships between sleep and body temperature cycles may play a key role in the oscillations between mania and depression in manic-depressives. An additional syndrome with links to altered circadian function is winter depression. The remission of the depression is simultaneous with the correction of the phase irregularity. Several lines of evidence demonstrate the sensitivity of the sleep control mechanism to the dynamic environment. The early Gemini flights showed changes in sleep duration and spectral power density of the electroencephalogram (EEG) early in the flight. On the Apollo and Skylab missions, sleep was also modified during initial exposure to space flight. Sleep onset has been a problem both for some Soviet cosmonauts and American astronauts, sometimes requiring the use of sleeping pills. Early reports on sleep stages assumed that slow wave sleep content is increased and REM sleep decreased. However, the recent Spacelab 1 findings of increased REM activity contradict this. There is a possibility that pre-flight sleep deprivation of Spacelab 1 subjects may have artificially increased REM sleep by well-documented rebound phenomenon. On a recent Mir mission, an individual showed a phase delay in this temperature rhythm and a diminished performance capacity that was linked to a decrease in fine motor control.

In summary, these investigations will provide basic information on function of homeostatic control systems in primates. This information will form the basis for the design of countermeasures used to prevent the performance, psychological, and health decrements that occur when these systems are adversely affected. These

countermeasures will not only be of the utmost importance as humans extend the length of time of exposure to space flight, but should also prove useful to people on Earth who suffer from homeostatic, particularly circadian, imbalances.

FY96 Publications, Presentations, and Other Accomplishments:

Fuller, C.A., Hoban-Higgins, T.M., Klimovitsky, V.Y., Griffin, D.W., and Alpatov, A.M. Primate circadian rhythms during spaceflight: Results from Cosmos 2044 and 2229. J. Appl. Physiol., 81 (1), 188-193 (1996).

Stein, T.P., Dotsenko, M.A., Korolkov, V.I., Griffin, D.W., and Fuller, C.A. Energy expenditure in rhesus monkeys (*Macaca mulatta*) during spaceflight using doubly labeled water (2H218O). J. Appl. Physiol., 81 (1), 201-207 (1996).

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

FY 1996 Funding: $31,200

Solicitation: NRA 88-OSSA-8

Expiration: 12/96

Students Funded Under Research: 4

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

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.

The study was not sufficiently developed to meet the constraints of the Bion mission, and therefore, was not flown.

FY96 Publications, Presentations, and Other Accomplishments:

Riley, D.A. Inflight and postlfight changes in skeletal muscles of rats flown in NASA Spacelabs and Cosmos Biosatellites. COSPAR Abstracts, (1996).

Riley, D.A., Ellis, S., Slocum, G.R., Sedlak, F.R., Bain, J.L.W., Krippendorf, B.B., Lehman, C.T., Macias, M.Y., Thompson, J.L., Vijayan, K., and DeBruin, J.A. Inflight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. J. Appl. Physiol., 81, 133-144 (1996).

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 1996 Funding: $200,010

Students Funded Under Research: 5

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

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).

With cooperation from our Russian colleagues, we trained rhesus monkeys in Moscow to perform several Psychomotor Test System (PTS) tasks. These monkeys readily learned to respond to computer-generated stimuli by manipulating a joystick. Baseline performance data were collected for each monkey, and behavioral indices of activity were also observed and scored. Two PTS-trained monkeys flew aboard the Bion 11 bio-satellite, launched in December of 1996 and recovered two weeks later. Scientists and technicians for the Behavior and Performance Project participated in this flight experiment with pre-flight and post-flight testing of the flight and control monkeys. Combined with ongoing ground-based investigations of rhesus monkey behavior and performance, we are confident that these data will contribute to a refined understanding of psychomotor control, cognition, psychological well-being, and the relation of each to physiological manifestations of adaptation to spaceflight.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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

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

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

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

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

Washburn, D. A. and Gulledge, J. P. The capacity of visuospatial memory for humans, apes, and monkeys. Psychonomic Society, Los Angeles, CA. November 1996.

Washburn, D.A. and Rumbaugh, D.M. Processing speed and intelligence: Is faster better? Psychonomic Society, Chicago, IL, November, 1996.

Washburn, D.A., Rogers, W.A., and Fisk, A.D. Attention: Principles and phenomena. Southern Society for Philosophy and Psychology, Nashville, TN. April 1996.

Washburn, D.A., Sevcik, R.A., Rumbaugh, D.M., and Romski, M.A. Educational applications of the Psychomotor Test System. Proceedings of the AIAA Life Sciences and Space Medicine Conference and Exhibit (pg. 72-73), American Institute of Aeronautics and Astronautics, 1996.

Washburn, D.A., Sevcik, R.A., Rumbaugh, D.M., and Romski, M.A. Educational applications of the Psychomotor Test System. AIAA Life Sciences and Space Medicine Conference and Exhibit, Houston, TX. March 1996.

Bone and Lean Body Mass Changes Following Space Flight

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Funding:

Project Identification:

Solicitation: NRA 88-OSSA-8

Initial Funding Date: 1/95

Expiration: 1/96

FY 1996 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

Flight Hardware Required: None

Task Description:

Issues of DEXA data interpretation with implanted electrodes and hardware were addressed through a series of measurements on control animals prior to collecting data on the flight animals. Skull region was subtracted from the analysis due to hardware. During FY 96 the first of two preflight DEXA mesurements were made on the 2 flight animals and ground control animals.

Previous flights involving animals and humans aboard Russian (Mir, Cosmos) and American spacecraft (Skylab, Spacelab) have documented that significant bone and muscle atrophy occurs during weightlessness requiring the development of effective and efficient countermeasures. The losses during space flight are believed to result from the reduced forces on the musculoskeletal system, analogous to the changes from inactivity in 1-G. The loss of bone mineral with aging occurs in both men and women, resulting in a significant public health problem in the United States and other countries of the world. It is estimated that the medical cost of osteoporosis in the 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.

Immunology Spaceflight and Immune Responses of Rhesus Monkeys

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Funding:

Project Identification:
Initial Funding Date: 10/90
FY 1996 Funding: $50,000

Solicitation: NRA 88-OSSA-8
Expiration: 10/99
Students Funded Under Research: 3

Flight Information:

Flight Assignment: Bion 11, 9/96
Responsible NASA Center: ARC

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 grant period, we completed analysis of data to prepare for a space flight of two rhesus monkeys. We perfected techniques for determination of interleukin production and leukocyte subset analysis of rhesus monkeys. Additionally, we completed analysis of our participation in the ARRT restraint test to determine if restraint conditions for flight in the space shuttle could contribute to any effects of space flight on immune responses. All immunological parameters listed in the methods section were tested. Evaluation of the data suggests that the restraint conditions had minimal effects on the results observed, but handling of the monkeys

could have had some effect. Also, to help us develop our rhesus monkey immunology studies, we carried out preliminary studies in mice to determine the effects of stressors on immunological parameters. We were able to show that there were gender-based differences in the response of immunological parameters to a stressor.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Morton, D.S., Swiggett, J.P., Hakenewirth, A.M., Fowler, N.A. and Sonnenfeld, G. Effects of movement limitation on immunological parameters. American Society for Gravitational and Space Biology, October, 1995.

Schmitt, D.A., Sonnenfeld, G., Husson, D., Tkaczuk, J., Andre, E., and Schaffar, L. *In vitro* interleukin-1 and -2 production and interleukin-2 receptor expression in the rhesus monkey. Life Sciences, 11, 931 (1996).

Adaptation to Microgravity of Oculomotor Reflexes

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Funding:

Project Identification:

Solicitation: NRA 88-OSSA-8

Initial Funding Date: 10/94

Expiration: 9/95

FY 1996 Funding: $

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Bion 11, 9/96

Responsible NASA Center: ARC

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 re-entry.

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 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.

Studies like these are necessary if there is to be continuing manned space flight. With a continuing presence of the U.S. 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.

This project was terminated in March 1996 due to a lack of funding from NASA.

Microgravity Effects on Bone Cell Gene Expression

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No Co-Is Assigned to this Task

Funding:

Project Identification: ARC-21129
Initial Funding Date: 2/95
FY 1996 Funding: $193,820

Solicitation: 93-OLMSA-07
Expiration: 2/97
Students Funded Under Research: 2

Flight Information:

Flight Assignment: Biorack, S/MM-03
Responsible NASA Center: ARC

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

Work Completed: We continued testing and activity plan for Biorack for the second year and completed the onflight experiment. (Feb 1995-Feb 1997):

1. Fixative 1: Leave fixative on modules with cells for specified times, freeze and test for recovery of RNA in samples using RNA 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, and 48 hrs, and 3,4, 6, and 8 days (compatible with material)

RESULTS: RNA recovered from cells grown and fixed in plunger boxers

2. 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 expression, if so, activiation will be delayed in orbit.

RESULTS: Testing started in spring 1996; one paper was published from initial studies. Only half completed, due to lack of funding for s/mm05 and 06. Still need to test long range effects of launch forces on gene expression.

3. Preparation and handover of samples for launch of STS-76 at KSC. Nominal ops and nominal recovery of sample RNA. Preliminary flight data looks promising.

1) mRNA expression during experimental protocol on c-fos, cyclophilin, COX 1 and 2, cPLA2
2) photography of cells fixed in formaldehyde using actin, Hoescht dye
3) rest of testing delayed due to lack of FY 96 funding for STS81 and 84, S/MM 05 and 06. Was forced to use money to cover costs of preparation of 05 mission. Without funding was unable to hire personnel for the preparation for 05.

RESULTS: Significant differences seen in microgravity and on board 1-G controls. Analysis will continue after STS-84 mission is completed.

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 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 an 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 (16). 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 (18). 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 (6). 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 (2,3) 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 add 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.

FY96 Publications, Presentations, and Other Accomplishments:

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

Hughes-Fulford, M. Growth regulation and gene expression in osteoblasts by prostaglandins. Proceedings of the International Conference on Eicosanoids and Other Bioactive Lipins in Cancer, Inflammation and Radiation Injury, 235-241, 1996.

Hughes-Fulford, M. and Lewis, M. Effects of microgravity on osteoblast growth activation. Exp. Cell Res., 224, 103-109 (1996).

Leong, J., Hughes-Fulford, M., Habib, A., Maclouf, J., and Goldyne, M. Cyclooxygenases in human and mouse skin and cultured human keratinocytes: Association of COX-2 expression with human keratinocyte differentiation. Exp. Cell Res., 224, 79-87 (1996).

Lewis, M.L. and Hughes-Fulford, M. "Cellular responses to microgravity" in "Textbook for the International Space University." Edited by: Churchill, Suzanne. Harvard University, Chapter 3, pp 71-105, 1996.

Graviperception in Starch Deficient Plants in Biorack

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 94-OLMSA-03

Initial Funding Date: 9/95

Expiration: 8/96

FY 1996 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: ARC

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.

In January 1997, our experiment PREPLASTID was launched on STS-81 as part of the European Space Agency's (ESA) Biorack payload. This experiment was a relatively small one and was in preparation for our larger main experiment PLASTID that is scheduled to be part of the Biorack payload on STS-84. In PREPLASTID, dry seeds of four strains of the plant *Arabidopsis* (wild-type, starchless mutant, and two reduced-starch mutants) were launched, and mission specialists activated the experiment by hydrating the seeds. These same four plant strains will be used on PLASTID.

Our accomplishments to date include:
1. From PREPLASTID, we determined that several changes in procedures had to be made to optimize the larger PLASTID experiment. Since the microgravity-grown seedlings grew at a slower rate than the ground controls, the stimulation times on the flight 1-G centrifuge had to be increased.

2. Also from the PREPLASTID experiment, we determined that the video camera should be zoomed in closer on the seedlings during PLASTID to better resolve both the roots and hypocotyls of the small *Arabidopsis* plants.

3. In the PREPLASTID experiment, condensation of water droplets on the plant growth chambers (minicontainers) was not a problem in microgravity as it was in some ground experiments.

4. Several preliminary scientific conclusions (in addition to the operational information listed above) can be drawn from the PREPLASTID experiment. (a) Growth rates of wild-type and the three mutant strains were reduced in microgravity compared to the ground. (b) Seedlings grown in microgravity had a higher density of root hairs relative to the ground controls. (c) Seedlings in microgravity had an abnormal development of their hypocotyl hooks. (d) Only hypocotyls of the wild-type seedlings responded to the 60 min 1-G stimulus given on the Biorack centrifuge during the spaceflight. The mutants (one starchless and two reduced starch) did not respond to this stimulus.

5. Working with ESA and KSC, we helped to design an illumination box that will be used on PLASTID. This device, which utilizes flight-approved red LEDs, will be used as a light source that will stimulate germination of the *Arabidopsis* seeds. During PREPLASTID, the Biorack glovebox light was used for seed germination. However, use of the LED illumination box will decrease the required illumination time from 14 hours to 10 minutes. This will simplify crew procedures and increases likelihood of a successful PLASTID experiment on STS-84.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Guisinger, M.M., Miller, A.J., and Kiss, J.Z. (abstract) The response to gravity is correlated to the amount of starch in *Arabidopsis* hypocotyls. American Society of Plant Physiologists, Midwestern Section, Urbana, Illinois, 1996.

Katembe, W.J., Edelmann, R.E., and Kiss, J.Z. (abstract) The development of spaceflight experiments with starch-deficient mutants of *Arabidopsis*. American Society for Gravitational and Space Biology, Charlotte, North Carolina. ASGSB Bull., 10, 46 (1996).

Kiss, J.Z. Immunolocalization of integrin-like proteins in *Arabidopsis* and *Chara*. Physiologia Plantarum, 99, 7-14.

Kiss, J.Z. Gravitropism in the rhizoids of the alga *Chara:* A model system for microgravity research. Marine Biological Laboratory meeting on planning for aquatic research in space. Woods Hole, Massachusetts. Abstract Book, p. 23. 1996.

Kiss, J.Z. Immunolocalization of integrins in *Arabidopsis* and *Chara*. American Society of Plant Physiologists, Midwestern Section, Urbana, Illinois. ASPP Midwest Abstract Book, p. 8.

Kiss, J.Z. and Swatzell, L.J. Development of the gametophyte of the fern *Schizaea pusilla*. J. Microscopy , 181, 213-221 (1996).

Kiss, J.Z., Guisinger, M.M., and Wright, J.B. What is the threshold amount of starch necessary for full gravitropic sensitivity? International Committee on Space Research (COSPAR). Meeting in Birmingham, England. COSPAR Abstract Book, p. 315.

Kiss, J.Z., Guisinger M.A., and Miller, A.J. What is the threshold amount of starch necessary for full gravitropic sensitivity? Advances in Space Research, (in press).

Kiss, J.Z., Guisinger, M.A., Miller, A.J., and Stackhouse, K.S. Reduced gravitropism in hypocotyls of starch-deficient mutants of *Arabidopsis*. Pl. & Cell Phys., 38, (in press).

Kiss, J.Z., Wright, J.B., and Caspar, T. Gravitropism in roots of intermediate-starch mutants of *Arabidopsis*. Physiologia Plantarum, 97, 237-244 (1996).

MacCleery, S.A., Meicenheimer, R.D., and Kiss, J.Z. (abstract) Plastid position in columella cells of starch-deficient mutants of *Arabidopsis*. American Society for Gravitational and Space Biology, Charlotte, North Carolina. ASGSB Bull., 10, 18 (1996).

Mechanisms of Gravity Sensing and Response in Hematopoietic Cells

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Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1996 Funding: $125,000

Joint Agency Participation: ESA

Solicitation: 93-OLMSA-07

Expiration: 5/97

Students Funded Under Research: 2

Flight Information:

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

Responsible NASA Center: ARC

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 STS-67 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; and 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.

The primary objective in 1996 was to fly the "TCELL" experiment on the STS-76 ESA Biorack Facility in March to evaluate cytoskeletal morphology, gene expression, signal transduction, and growth response in Jurkat cells. Ten "Cytokines" cassettes, five for static microgravity and five for the 1.4-G inflight centrifuge were set up along with the comparable ground controls. Each cassette consisted of six wells with approximately 700,000 cells, six wells with culture medium containing a high serum concentration to activate cell growth on orbit, and six medium reservoirs to collect conditioned medium filtered from cell wells. Cells were fixed with 3% formalin at 4 and 48 hours for cytoskeletal morphology, flow cytometry, and nuclear characteristics. Cells for evaluation of genes regulating growth and cytoskeletal morphology were lysed at 4, 24, and 48 hours with guanidinium isothiocyanate (GITC). Formalin fixed cells were held at 5°C and GITC cells were stowed at -20°C for the remainder of the mission.

Evaluations in 1996 focused on cytoskeletal morphology and cellular responsiveness to growth stimulation. Immunofluorescence microscopy and laser scanning confocal microscopy showed a striking difference in microtubule organization between flown and ground cells. Flown cells and diffusely organized microtubule structures extending from dispersed organizing centers (MCTOCs). In comparable ground controls the cytoskeletal microtubule complex radiated in discrete branches to the membrane. This observation points to cytoskeletal alterations as a primary mechanism for reduced growth responsiveness in flown hematopoietic cells. The flown cell population did not increase in number whereas the ground controls almost doubled. However, the DNA profiles in propidium iodide stained cells evaluated by flow cytometry indicated cell cycle traverse in a sub-population of the flown cells. Glucose utilization confirmed metabolic function and was almost twice that of ground controls. The observed zero population increase in flown cells may be explained in part by the higher incidence of apoptosis. In 1997, research will focus on further evaluations of the TCELL samples for apoptosis, gene expression and signal transduction markers. The effect of launch vibration and acceleration forces on cytoskeletal morphology and gene expression will also be evaluated in ground-based testing.

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

FY96 Publications, Presentations, and Other Accomplishments:

Clunie, J.C., Lewis, M.L., Albright, D.T., and Baird, J.K. "A search for gravitational effects in diffusion" in "Space Processing of Materials." SPIE- The International Society for Optical Engineering, 2809, pp 244-246, 1996.

Hughes-Fulford, M. and Lewis, M.L. Effects of microgravity on osteoblast growth activation. Exp. Cell Res., 224 (1996).

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

Lewis, M.L. and Hughes-Fulford, M. "Cellular responses to microgravity" in "Fundamentals of Space Biology." International Space University, Krieger Publishing Co., Melbourne, FL., 1996.

Modification of Radiogenic Damage by Microgravity

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93-OLMSA-07

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: ARC

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.

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Bacterial Growth on Surfaces in Microgravity and on Earth

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Co-Investigators:

Gordon A. McFeters, Ph.D.; Montana State University

Funding:

Project Identification:

Solicitation: 94 OLMSA-03

Initial Funding Date: 7/95

Expiration: 6/98

FY 1996 Funding: $79,065

Students Funded Under Research: 2

Flight Information:

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

Responsible NASA Center: ARC

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.

Preliminary experiments on biocompatability and evaluation of incubation conditions continued through 1996. Two undergraduate student assistants started work on the project in January 1996 and continue to the present. The Experiment Sequence Test was completed at Kennedy Space Center in May. The results were summarized in the annual project report. In December, filled containers were shipped to Ames Research Center for vibration and acceleration tests under the supervision of Dr. Ron Schaefer. The results suggested some possible effects, which require further investigation. Preparation for the flight experiment was mostly completed by the end of 1996 in readiness for an early January departure to Kennedy Space Center.

Preliminary data from the flight experiment (January 1997) suggests that the biofilms grew about as well or slightly more rapidly in microgravity as in the space flight centrifuge 1-G and Earth-based controls. Thus, it

should be possible to utilize surface-associated, immobilized bacterial cultures for the production of specific novel compounds in spacecraft.

FY96 Publications, Presentations, and Other Accomplishments:

Huang, C.-T., McFeters, G.A., and Stewart, P.S. Evaluation of physiological staining, cryoembedding, and autofluorescence quenching techniques on fouling biofilms. Biofouling 9:269-277. 1996.

McFeters, G.A., Pyle, B.H., and Broadaway, S.C. Detection of specific respiring bacteria in water using immunomagnetic separation combined with cyanoditolyl tetrazolium chloride. American Water Works Association Water Quality Technology Conference, Boston, MA, November, 1996.

Pyle, B.H., and McFeters, G.A. (abstract) Detection of respiring *E. coli* O157:H7 using immunomagnetic separation, cyanoditolyl tetrazolium chloride, and a fluorescent antibody. American Society for Microbiology 96th General Meeting, New Orleans, LA, Q-439, p. 462, May 1996.

Smith, J.J., and McFeters, G.A. Effects of substrates and phosphate on INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride) and CTC (5-cyano-2,3-ditolyl tetrazolium chloride) reduction in *Escherichia coli*. J. Appl. Bacteriol. 80:209-215. 1996.

Wentland, E.J., Stewart, P.S., Huang, C.-T., and McFeters, G.A. Spatial variations in growth rate within *Klebsiella pneumoniae* colonies and biofilm. Biotechnol. Prog. 12:316-321. 1996.

Gravitropism and Autotropism in Cress Roots

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Funding:

Project Identification:

Solicitation: NRA 94-OLMSA-03

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 1

Joint Agency Participation: ESA

Flight Information:

Flight Assignment: Biorack

Responsible NASA Center: ARC

Flight Hardware Required: Biorack, photobox

Task Description:

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

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

Our progress is best summed up by including excerpts of two abstracts that will be presented at the American Society for Plant Physiology meetings in July, 1997.

I. Autotropic straightening after gravitropic curvature of Lepidium roots

Space flight experiments have shown that roots that are laterally centrifuged and then returned to microgravity first curve and then undergo "autotropic straightening." But this phenomenon has not been well documented in ground-based experiments using a clinostat. Vertically-grown cress (*Lepidium sativum L.*) roots were turned to the horizontal for 1 to 5 h and then rotated at 1 rpm on a clinostat. The axis of clinostat rotation was either parallel (axial) or perpendicular (somerault) to the long axis of the root. The kinetics of root curvature were monitored following placement on a clinostat. Roots that had been horizontal for 1 h continued to curve for a while when placed on a clinostat, but later the curved regions straightened. The magnitude of root tip straightening was 20-30. Roots that were horizontal for 5 h also showed straightening of curved tip regions, but the basal part of the root continued to curve in the direction of the most recent stationary G-stimulus. Autotropic straightening occurred regardless of whether roots were rotated in an axial or somersault configuration. Control roots, which were vertical roots placed directly on a clinostat, grew at a slightly skewed angle after rotation on the clinostat. This slanting was also seen in horizontally-stimulated roots and in both cases had a magnitude of 3-8, and was uniformly manifested over the entire length of the root. These data show that gravitropic curvature can be lost upon removal of the gravity stimulus through exposure to omnilateral stimulation on a clinostat thus validating the phenomenon of autotropic straightening. This suggests that gravitropic curvature is not necessarily permanent and that the root retains some sort of "memory" of its orientation prior to gravitropic stimulation.

II. Autotropism, automorphogenesis, and gravity

Segments of organs that have undergone gravitropic curvature later straighten during the course of gravitropism or after the G-vector becomes randomized on a clinostat. Little is known about the mechanisms underlying these and perhaps related phenomena which have been described with various overlapping terms such as autotropism, autotropic straightening, automorphosis, automorphogenesis, automorphic curvature, and gravitropic straightening. The types of phenomena that historically have been named by the above terms are reviewed critically with respect to an interaction with gravitropism. We suggest that the term "autotropism" should not be applied to the phenomenon of organ straightening that occurs during the course of gravitropism, since this straightening is part of a complex series of local growth adjustments overall through time and since this phenomenon is not itself a tropistic response to a directional exogenous stimulus. It is suggested that the term autotropism should be used only for the phenomenon of organ straightening that occurs after the G-vector is randomized on a clinostat or withdrawn in the microgravity conditions of space flight. Usage of the term automorphogenesis is most appropriate for describing curvatures or orientations that result from morphological relationships such as in nastic curvatures.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Geisler, M., Yang, M., and Sack, F.D. (abstract) TMM differentially affects stomatal precursor cell formation and activity in *Arabidopsis* organs. *Arabidopsis* meeting at University of Wisconsin, June 1995.

Kern, V.D. and Sack, F.D. (abstract) Gravitropism and phototropism in *Ceratodon purpureus*. ASGSB Bull., 10, 97 (1996).

Nadeau, J.A., Geisler, M., and Sack, F.D. (abstract) Genetic dissection of stomatal development in Arabidopsis. FASEB Plant Developmental Biology Conference, Saxtons River, VT. 1996.

Sack, F.D. and Schwuchow, J. (abstract) Protonemal gravitropism and amyloplast sedimentation in the mosses *Funaria* and *Physcomitrella.* Amer Soc Gravitational Space Biol Bull, 9, 140 (1995).

Sack, F.D., Schwuchow, J., and Kern, V.D. (abstract) Gravitropism in high density media supports intracellular, statolith-based sensing in moss protonema (*Ceratodon*). ASGSB Bull., 10, 99 (1996).

Sack, F.D., Yang, M., and Geisler, M. (abstract) Mutational analysis of stomatal development in *Arabidopsis*. J. Cellular Biochem. Suppl., 21A, 440 (1995).

Wagner, T.A., Cove, D.J., and Sack, F.D. "A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon.*" in "Plants in Space Biology." Edited by: Suge, H. Institute of Genetic Ecology, Tohoku University, Japan, pp 53-60, 1996.

Wagner, T.A., Cove, D.J., and Sack, F.D. (abstract) A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon*. ASGSB Bull., 10, 98 (1996).

Wagner, T.A., Schwuchow, J., Oakley, C.E., Oakley, B.R., and Sack, F.D. (abstract) Isolation and characterization of a -tubulin cDNA from the moss *Physcomitrella patens*. Pl. Physiol. Suppl., 108, 585 (1995).

Yang, M. and Sack, F.D. The too many mouths and four lips mutations affect stomatal production in *Arabidopsis*. Plant Cell, 7, 2227-39 (1995).

Yang, M. and Sack, F.D. (abstract) Analysis of phenotypes of stomatal cluster mutants in *Arabidopsis* cotyledons. *Arabidopsis* meeting at University of Wisconsin, June, 1995.

Yang, M., Nadeau, J., and Sack, F.D. (abstract) Characterization of a cytokinesis defective (*cyd*) mutant in *Arabidopsis*. Amer. Soc. Cell Biol., Abstract No. 1912, in Mol. Biol. Cell, Suppl. 7, 329A (1996).

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 1996 Funding: $
Students Funded Under Research: 3

Flight Information:

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

Responsible NASA Center: ARC

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 of 1) the formation of intercellular signaling complexes; and 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.

Modifications to the AGGREGATE hardware were completed in FY96. The modifications enabled the introduction of small (6-10 micron) beads into the culture chambers during flight to activate the T cells. This modification was tested in two mission simulations at the Kennedy Space Center. Detailed crew procedures,

experiment timelines and flight documentation were prepared for this experiment, and all certification of hardware and experiment parameters was completed. Ground-based verification tests were also perfomed in the laboratory to verify perfomance of all aspects of the experiment and to provide ground control data. The experiment is currently manifested on STS-81 and STS-84 which will fly in FY97.

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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Funding:

Project Identification:

Solicitation: 94 OLMSA-03

Initial Funding Date: 9/95

Expiration: 8/96

FY 1996 Funding: $93,568

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: ARC

Task Description:

The overall goal of the project is to determine whether second messenger signal transduction pathways in sperm are altered in microgravity compared to Earth-normal gravity. A previous experiment which examined a limited number of sperm movement parameters demonstrated that bovine sperm motility is altered significantly in microgravity. Prior to that study, exposure of sperm to hypergravity was found to produce a dramatic decline in the content of ATP and a rise in ADP in sperm; motility in these experiments was not determined. In this connection, sperm able to swim against a 1-G force were found to contain higher levels of ATP. Since ATP is critical to sperm motility, it is likely that microgravity may produce changes in sperm motility. Sperm motility is regulated by the content of cAMP, calcium ions, and the state of protein phosphorylation modulated by these second messengers. Whether these components are altered during changes in gravitational forces is not known. The present study will examine changes in protein phosphorylation in sea urchin under a variety of physiological and gravitational conditions, including those which promote oocyte fertilization on the ground in the presence and absence of speract, a chemotactic peptide from sea urchin eggs. We will also examine if protein phosphorylation in sperm activated in microgravity in sea water made with deuterium oxide (heavy water) is similar to activation at 1-G in normal sea water. The project will also yield information regarding biochemical mechanisms underlying the alterations in movement produced by alterations in gravity. Results from these experiments will greatly expand previous data regarding the effects of altered gravity on sperm function and early fertilization events. The flight experiments for this project will be carried out on Shuttle/MIR docking flights STS-81 (S/MM-05) which launched January 12, 1997, and STS-84 (S/MM-06) scheduled for May 15, 1997.

The experiment on STS-81 Biorack (SPERM) went extremely well. The sperm preparation for hardware loading was excellent. Video microscopic analysis of replicate ground samples simultaneous to performance of the flight experiment indicated that the sperm in the hardware was absolutely immotile during the pre-experiment activation period and that 90% motility was achieved upon activation by injection of sea water. All of the manipulations by Mission specialist John Grunsfeld were nominal. Analysis of the flight and ground-control data has begun. Initial western immunoblot data suggests that 1) there were differences in the rate of phosphorylation of proteins in the sperm activated in microgravity and 2) some proteins increased during

activation while others decreased during activation in microgravity. The simulation for STS-84 was conducted, and modifications in the experiment timeline were made. Analysis of the data for the simulation has also begun. Preparations for STS-84 (SPERM-A) are well underway and should yield another successful experiment.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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

Effect of Space Flight on Adrenal Medullary Functions

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Project Identification: BSP-012
Solicitation: 93-OLMSA-02
Initial Funding Date: 1/95
Expiration: 12/95
FY 1996 Funding: $
Students Funded Under Research: 2

Flight Information:

Responsible NASA Center: ARC

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.

Background: A major focus of our laboratory is the study of stimulus-secretion coupling in adrenal medullary chromaffin cells. In participating in the Tissue Sharing Program, the specific aim of our initial studies was to

analyze the effects of space flight on adrenal medullary function in rats flown aboard STS 54. The data, obtained from 6 animals flown for 10 days in space and 6 age/sex matched controls, suggested that microgravity decreased the expression of tyrosine hydroxylase (TH), the rate- limiting enzyme of catecholamine synthesis, at the levels of gene and protein expression as well as at the level of enzyme activity. By contrast, the expression and enzymatic activity of phenylethanolamine-N-methyl transferase (PNMT), the key enzyme in the conversion of epinephrine to epinephrine, were not affected by microgravity. The results of these studies were reported in a peer-reviewed paper in FASEB J.

In order to further validate these findings we responded to 93-OLMSA 02 and applied in July 1993 for participation in the Tissue Sharing Program with the aim to evaluate additional adrenals from rats flown on different missions and dissected either during space flight or upon return to Earth. After favorable peer-review, a grant (NAG-9-71) was awarded on 3/24/1994. Unfortunately, funding for this grant did not commence until 4/1/1995. Moreover, the specimens (approx 300 glands from PARE.03 and SLS-2) were not shipped to our facility until 12/15/1995.

Progress (January 1, 1996 - March 31, 1997): Given the limited manpower and modest funds available, we have analyzed in the past 15 months 32 samples (approx 10 % out of a total of approx. 300 samples). Using largely biochemical and immunological techniques, we evaluated the expression of various catecholamine synthesizing enzymes (CASE): a) tyrosine hydroxylase, TH, the rate-limiting enzyme, a) dopamine- -hydroxylase (DBH), which catalyzes the conversion of dopamine into norepinephrine, and c) PNMT, the enzyme which catalyzes the formation of epinephrine from nor-epinephrine. At this time, we have not yet performed catecholamine anlyses by HPLC-EC, nor have we as yet analyzed the steady state mRNA expression, which are the two most labor-intensive assays. Rather, for our preliminary experiments we evaluated CASE expression and activity by Western blotting, and enzyme assays, respectively. In addition, total catecholamines were determined biochemically. In our initial studies we used specimens which were comparable to those obtained from PARE.02, viz., adrenals from flight animals sacrificed within the first 5.5 h after return from space flight, as well as the appropriate controls (viz. vivarium, synchronous controls, and tail- suspended animals) .

We have, so far, analyzed two samples from each of these groups. In brief, adrenals were homogenized as described (Lelkes et al, 1994) and analyzed for the expression of immunoreactive CASE levels (Western blotting) as well as for CASE activities (radioenzymatic assays). Aliquots of the homogenates were also assayed for total catecholamines using an established fluorescent method.

To summarize our findings to-date, the preliminary data from both PARE.03 and SLS-2 are significant for several reasons: 1) in spite of the limited number of samples analyzed, the results seem to confirm our previous findings, namely that the expression of TH, but not of PNMT is impaired during space flight; 2) as a novel finding, these preliminary data also suggest that the expression of DBH is not affected by microgravity in space; 3) microgravity in space seems to have the opposite effect on adrenal medullary function than a ground-based model for "simulated microgravity," viz. tail suspension: both the expression and activities of TH and PNMT increase in tail-suspended rats, while according to our preliminary data, the expression/activity of DBH may be decreased; and 4) last but not least, our preliminary findings put to rest legitimate concerns regarding the state of preservation of the samples. Our preliminary results prove the feasibility of our approach and we are looking forward to completing our sample analysis.

Future plans: The remaining funds from the current grant will be used during the coming six months for purchasing reagents and for hiring a summer student who will participate in further evaluating the existing samples by biochemical and molecular biological techniques. A new proposal in response to HEDS-04 has been submitted. If and when this proposal is funded, we will proceed with the full scale analysis of our valuable specimens.

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.

Vitamin D Endocrine System After Short-term Space Flight

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Project Identification: BSP-006
Initial Funding Date: 9/94
FY 1996 Funding: $29,610

Solicitation: 93-OLMSA-02
Expiration: 12/95
Students Funded Under Research: 2

Flight Information:

Responsible NASA Center: ARC

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 nine days aboard the PARE.03 mission. The effects of microgravity on calbindins in rats in space were compared with synchronous Animal Enclosure Module controls, modeled weightlessness animals (tail suspension), and their controls. Exposure to microgravity resulted in a significant decrease in calbindin-D_{28k} content in kidneys and calbindin-D_{9k} in small intestine, as measured by enzyme-linked immunosorbent assay (ELISA). Modeled weightlessness animals exhibited a similar decrease in calbindins by ELISA. Immunocytochemistry (ICC) in combination with quantitative computer image analysis was used to measure *in situ* the expression of calbindins in the kidney and the small intestine, and the expression of insulin in pancreas. There was a large decrease of immunoreactivity in renal distal tubular cell-associated calbindin-D_{9k} of space flight and modeled weightlessness animals compared with matched controls. No consistent differences in pancreatic insulin immunoreactivity between space flight, modeled weightlessness, and controls were observed. Regression analysis of results obtained by quantitative ICC and ELISA for space flight, modeled weightlessness animals, and their controls demonstrated a significant correlation. These findings suggest that a decreased expression of calbindins after short-term exposure to microgravity and modeled weightlessness may affect cellular Ca^{2+} homeostasis and contribute to disorders of Ca^{2+} and bone metabolism disorders induced by space flight.

Two calcium-binding proteins, calbindin-D_{28k} and calbindin-D_{9k}, were measured in rat tissues from flight, simulated weightlessness, and control animals using ELISA with monocolanal anti-calbindin-D_{28k} antibodies and polyclonal antiserum against calbindin-D_{9k}. Significant decreases in levels of renal calbindin-D_{28k} and intestinal calbindin-D_{9k} were found in flight and simulated weightlessness animals. The level of renal calbindin-D_{9k} was very low and only changed signficantly in immediatedly postflight animals.

Immunocytochemistry in combination with quantitive computer-based image analysis was used to measure *in situ* levels of calbindins on fixed tissues. The primary antibodies described above and an antibody against insulin were used. Results were similar to those obtained with ELISA, with declines in calbindins in kidney and

intestine in flight and tail suspension groups. Insulin levels in pancreatic beta-cells varied greatly within groups.

In summary, the tasks completed produced results demonstrating decreased expression of calbindins after a short-term (nine-day) exposure to microgravity and modeled weightlessness. The declines in calbindins may affect cellular Ca^{2+} homeostasis and contribute to the develoment of Ca^{2+} and bone metabolism disorders induced by space flight. Whether or not 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 regulation and stabilization of Ca^{2+} and the genesis of microgravity-induced disorders of calcium metabolism. Intracellular Ca^{2+} is a nearly universal second messenger and many cellular processes depend upon calcium including muscle contraction, bone formation and remodeling, cell division, programmed cell death (apoptosis), and gene expression. Our understanding of regulation of cells, both normal and abnormal (for example, cancer cells), at normal Earth gravity can be enhanced. Understanding of the mechanisms underlying disorders of calcium, metabolism are important to the development of pharmacologic and dietetic therapeutic strategies to prevent bone and calcium metabolic diseases in space and on Earth.

FY96 Publications, Presentations, and Other Accomplishments:

Rhoten, W.B., Sergeev, I.N., Chaudhry, M.A., and Carney, M.D. Effects of short-term space flight on calbindins. XXVI Congress of the Anatomical Society of Southern Africa, 1996.

Sergeev, I.N., Carney, M., Chaudhry, M.A., and Rhoten, W.B. Calcium-binding proteins reduced in short-term space flight. Zimbabwe Association of Clinical Pathologists and Medical Scientists, 1996.

Sergeev, I.N., Rhoten, W.B., and Carny, M.D. Calbindins decreased after space flight. Endocrine, 5, 335-340 (1996).

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
Solicitation: AO/Cosmos Program
Initial Funding Date: 1992
Expiration: 1995
FY 1996 Funding: $
Students Funded Under Research: 4

Flight Information:

Responsible NASA Center: ARC

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 in 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

which were fed commercial diets heavily supplemented with vitamin D and in Russian IMBP which were 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 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 content. 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.

FY96 Publications, Presentations, and Other Accomplishments:

Hutchinson, T.M., Bakulin, A.V., Rakhmanov, A.S., Martin, R.B., Steele, C.R., and Arnaud, S.B. Effect of chair restraint on the mechanical properities of tibias in Rhesus monkeys. Calcified Tissue Intl., (in press).

Roberts, S.G., Hutchinson, T.M., Arnaud, S.B., Kiratli, B.J., Martin, R.B., and Steele, C.R. Non-invasive determination of bone mechanical properties using vibration response; A refined model and validation *in vivo*. J. Biomechanics, 29, 91-98 (1996).

Effects of Microgravity on Quail Eye Development

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 5/96

FY 1996 Funding: $60,302

Solicitation: 93-OLMSA-06

Expiration: 4/97

Students Funded Under Research: 2

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

Task Description:

We propose that embryonic eye development will occur normally in microgravity. Our studies of chicken embryo eyes from STS-47 (flight vs. controls) have indicated no major differences, except that in the cornea, a much higher than normal percentage of the central cornea area contains cellular processes (pseudopodia, filopodia, long blebs, or neurites) inthe extracellular matrix of Bowman's Layer. These inclusions were not seen in corneas of Day-14 or Day-17 embryos but are prominent in the corneas of hatchlings that came from embryos that flew in space. To confirm these results from chickens by making corresponding observations in quail, we propose to examine the eyes of quail embryos that flew in space (and corresponding ground controls). We will examine eyes and corneas from embryonic Day-16 (E16) quail. One aspect of our investigation will be to look for the possible occurrence of abnormal numbers of cellular processes in the central areas of corneas in that region of Bowman's Layer just beneath the basal lamina of the corneal epithelium (the most anterior portion of the corneal stroma). We will observe nerve development patterns in the corneas, examine corneas for clarity, and observe physical parameters of eyeballs, corneas, and the ossicle (bone) rings surrounding the corneas.

We observed whole eyes and corneas from E16 quail embryos, and eyes from E14 embryos. The following eye measurements were recorded: 1) eyeball weight; 2) eyeball diameters (dorsal/ventral and nasal/temporal); 3) corneal diameters (dorsal/ventral and nasal/temporal); and 4) inner and outer ossicle ring diameters (dorsal/ventral and nasal/temporal).

Corneal transparency was documented by photographing a fine wire mesh viewed through the corneas. Corneas were removed, and the scleral ossicle ring was stained to observe bone numbers and orientation. A wedge-shaped section was removed from the ventral region of each cornea and prepared for transmission electron microscopy. Remaining corneal tissue will be stained immunohistochemically with antibodies against neurofilaments to detect corneal nerve patterns using a whole-mount procedure devised this year in our lab (Barrett et al., in preparation). Group means were compared statistically to determine if there are differences in the means for the three treatment groups (Flight, Synchronous Control, and Lab Control) for all observed physical parameters. Quantitative and qualitative observations indicate that our original hypothesis is supported, since we found no significant differences between Flight eyes and corneas compared with those of either of the control groups.

A question has arisen during our work on the tissue from this experiment concerning the quality of fixation. We found the tissue from all three groups (Flight, Synchronous, and Ground Controls) to be poorly fixed, particularly when observed at the electron microscope level.

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

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

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

FY96 Publications, Presentations, and Other Accomplishments:

Barrett, J.E., Wells, D.C., and Conrad, G.W. Increased neurofilament immunostaining in highly fixed embryonic quail corneas after microwaving, sodium dodecyl sulfate incubation, and hyaluronidase incubation. Sunflower Developmental Genetics Symposium. Kansas University Medical Center, Kansas City, KS, September 28-29, 1996.

Skeletal Development in Long Duration Spaceflight

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 6/95

FY 1996 Funding: $64,816

Solicitation: NRA 93-OLMSA-06

Expiration: 5/97

Students Funded Under Research: 1

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

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.

Tissue Collection:

Skeletal samples included wing, leg (tibia and femur), mandible, ribs, and spine collected by dissection at Ames, October 7-12, 1996. From the Flight groups, 4 embryos out of 12 eggs were viable at D16, 5 out of 8 at D14, 5 out of 8 at D10, and 5 out of 8 at D7. The Synchronous Controls provided a slightly better yield, whereas the Lab Control embryos were nearly 100% developed.

Tissue Preparation and Results:

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

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

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

Mandibles: Mandibles were embedded in Spurr's resin and sectioned for light microscopy. Some sections were analyzed by x-ray microscopy to produce Ca/P ratios. The results indicate normal mineral ratios in the 16D controls and flight samples, but the ratio in the 10D flight was reduced compared to its control. This data is being compared to similar data collected from 10D, 14D, and 16D long bones and wing bones to see if the effect is "real" (not a technical artifact) and if it is a generalized result found in the whole skeleton.

By light microscopy, we demonstrated the mineralized bone in the mandibles of the D10 embryos. It appears that there is less mineral present in the D10 flight compared to the synchronous controls. This would suggest, like the Ca/P data described above, that bone formation at D10 is sensitive to space flight conditions.

Infrared (IR) microscopy is being used to analyze the mineral chemistry and the relative collagen to mineral ratios in the sections of mineralized bones. Again, the D10 samples varied from the spectra of the D14 and D16 embryonic mandibles. It appears that the phosphate peak in the D10 flight samples is larger and broader than this peak from the D10 synchronous control. This may be due to the presence of pyrophosphate which can alter the chemistry and solubility of the bone mineral. This finding needs further study and analysis from other D10 bone samples. We also evaluated the mineral to organic matrix content with IR microscopy. This ratio showed no difference between flight and control at D14 and D16. However, at D10, the flight sample was significantly different from the D10 control. This difference appears to be due to alteration in the mineral content. Again, this finding needs confirmation from analysis of other bone samples (eg. tibia, femur, wing, etc).

Preliminary Findings:

There appears to be a disturbance of mineral chemistry and/or mineralization of mandible bone, in the D10 Flight samples which is not present in the D10 Controls or in the D14 and D16 Flight or Control samples. This result needs to be verified in other skeletal tissues to see if it is a generalized effect. If this did occur during flight, then apparently the embryo overcame this problem by D14/D16. We therefore need to study the D10 group in much more detail, especially looking at the mineralization process, cellular activity during bone formation, and mineral deposition in calcifying cartilage compared to bone.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Boskey, A.E., Guidon, P., Doty, S.B., Stiner, D., Leboy, P., and Binderman, I. The mechanism of beta-glycerophosphate action in mineralizing chick limb-bud mesenchymal cell cultures. J. Bone Mineral Res., 11, 1694-1702 (1996).

Doty, S.B. and Dicarlo, E.F. Pathophysiology of immobilization osteoporosis. Current Opinion in Orthopedics, 6, 45-49 (1995).

Moursi, A.M., Damsky, C.H., Lull, J., Zimmerman, D., Doty, S.B., Aota, S.-I., and Globus, R.K. Fibronectin regulates calvarial osteoblast differentiation. J. Cell Sci., 109, 1369-1380 (1996).

Effect of Microgravity on Afferent Innervation

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Funding:

Project Identification:

Initial Funding Date: 9/95

FY 1996 Funding: $

Solicitation: NRA 93-OLMSA-06

Expiration: 9/96

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-MIR

Responsible NASA Center: ARC

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 quail (*Coturnix coturnix japonica*) raised in space.

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

II. On the opposite ear and brain stem of each animal (ground or flight), the neurofilament protein (NF), the S-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 µm2 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.

The research proposed in this application does not seek to develop new therapeutic treatments for use at the 1-G Earth environment. The research will, however, provide invaluable information for better understanding the functioning of the vestibular (balancing and equilibrium) system in vertebrates. Even today, after decades of space exploration, astronauts suffer vestibular disturbances in microgravity despite intense and sophisticated training before space flights. The main reason for this is that at 1-G, certain conditions of the space environment can not be replicated for a long period of time. Only long-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-G are known to depend stimulation aided by the force of the gravity vector. There are sufficient data published in peer-reviewed journals to indicate that otoconia found in the inner ear of vertebrates may influence the bearing load upon hair cells that lead to their depolarization and initiation of vestibular stimulus. However, we know nothing about the effect that microgravity may play in the development of otoconia when the animal is permitted to develop in microgravity from the time of conception.

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

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

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

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Effects of Weigtlessness on Vestibular Development in Quail

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Funding:

Project Identification: 106-31-01
Solicitation: 93-OLMSA-06
Initial Funding Date: 7/95
Expiration: 6/97
FY 1996 Funding: $91,395
Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir
Responsible NASA Center: ARC

Task Description:

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

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

The tasks at hand could not be completed simply because of lack of viable quail around the time needed. Thus far, our total of quail received is at 4 microgravity-exposed animals for the two days. Clearly, no conclusions can be drawn on this limited sample size, and we are awaiting further animals to complete the analysis.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Bianchi, L.M., Conover, J.C., Frizsch, B., De Chiara, T., Lindsay, R.M., and Yancopoulos, G.D. Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. Development, 122, 1965-1973 (1996).

Fritzsch, B. Development of the labyrinthine efferent system. Ann. N.Y. Academy of Sci., 781, 21-33 (1996).

Fritzsch, B. How does the urodele ear develop? Int. J. Dev. Biol., 40, 763-771 (1996).

Fritzsch, B. Similarities and differences in lancelet and craniate nervous systems. Israel J. Zool., 42, 147-160 (1996).

Fritzsch, B. and Hallbook, F. A simple and reliable technique to combine oligonucleotide probe *in situ* hybridization with neuronal tract tracing in vertebrate embryos. Biotech. & Histochem., 71, 289-294 (1996).

Hellmann, B. and Fritzsch, B. Neuroanatomical and histochemical evidence for the presence of common lateral line and inner ear efferents and of efferents to the basilar papilla in a frog, *Xenopus laevis*. Brain, Behavior and Evolution, 47, 185-194 (1996).

Hypogravity's Effect on the Life Cycle of Japanese Quail

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: NRA

Initial Funding Date: 6/95

Expiration: 5/96

FY 1996 Funding: $ 50,134

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

Task Description:

The quail eggs imported from Moscow were found to be free of disease and were released from quarantine; however, due to late shipment by the Russians, birds did not reach sexual maturity in time of the launch of STS-76. Instead a random bred strain of Japanese quail eggs from the Poultry Scicence Department of the University of Wisconsin (Dr. Berndard Wentworth) were launched.

Egg shells from flight (STS-76, MIR 21-NASA 2) and control quail embryos of days 3, 7, 10, 14, and 16 of incubation have been analyzed for shell mineral content. Flight embryos used significantly less calcium from the shell when compared to both synchronous and laboratory controls. Space flight conditions interfered with the 16 day-old quail embryos' uptake of calcium from the shell. However, since synchronous control embryos at day 16 had shell calcium levels that did not differ statistically from those in space flight, the effect may have been due to the high incubator temperature (39° to 40° C) experienced during space flight as opposed to microgravity. The new question that has arisen is whether higher than normal incubator temperatures affect the utilization of calcium from the shell. This is the first year and mission (Mir 21 - NASA-2) in which adequate sample size has been obtained from flight quail embryos to conduct laboratory and statistical analysis. Future work necessitates more reliable incubation hardware in microgravity.

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

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

FY96 Publications, Presentations, and Other Accomplishments:

Fermin, C. D., Martin, D, Jones, T., Vellinger, J., Deuser, M., Hester, P., and Hullinger, R. Microgravity in the STS-29 space shuttle Discovery affected the vestibular system of chick embryos. Histol. Histopath., 11, 407-426 (1996).

Avian Blood Formation in Space

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Funding:

Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $

Students Funded Under Research: 1

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

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.

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 flight (quoted in the NRA 93-OLMSA-06, Appendix A, page 2). 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.

The objective of our research is to test the hypothesis that exposure to microgravity during space flight cause delayed or improper vascular development during embryogenesis. The effects of microgravity on the time course and extent of avian blood-vessel formation will be assessed using two models, one for angiogenesis and one for vasculogenesis. The methodological approach is dictated by the constraints of the tissue preservation method used in space. Thus, both in the chorioallantoic membrane and in the adrenal, we will 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.

Analysis of the from Mir 19/ STS-74 during the first 9 months of 1996 yielded little useful tissue from the flight samples and the synchronous controls, while the ground laboratory controls appeared to be normal. The flight samples and two sets of ground controls form NASA2/Mir 21 were dissected between October 6 and 20, 1996 at ARC. Initial observations at the time of dissection included the number of samples, and their developmental stages. Upon dissection, the gross appearance of the chorioallantoic membrane (CAM) was noted. The samples were shipped on November 4, 1996 to our laboratory

There were altogether "useful" 68 CAMs from Mir 21, which appeared "intact" as inferred by gross visual inspection and which are currently being further analyzed. Since November 1996, we have provisionally examined a total of 36 CAMs by light microscopy looking at three samples from each group, i.e., CAMs from three different sets (flight synchronous controls, ground controls) and four time points (E 7, E 10, E 14, E 17). For these studies we are using two established methods, which previously gave us quantifiable data: a) en face

bright field/fluorescence microscopy to delineate at low power magnification vascular morphology, and b) immunohistochemistry for highlighting the structure of the blood vessels.

Although the number (n=3) for each set is too small to make any conclusive statements, we made the following preliminary observations:

1) The development of the CAMs, as inferred from vessel density and vessel size, seems to be somewhat retarded in the flight controls, as compared to the ground controls;
2) In contrast to our previous finding (based on analysis of MIR 18 samples), there does not seem to be a profound effect of the "flight-simulating conditions" on the development of the CAM in the synchronous controls. The CAMs from the synchronous control embryos are virtually indistinguishable from those in the ground controls; and
3) Based on the small number of samples examined so far, we seem to encounter some inconsistencies regarding the fixation of the samples. In all three groups (flight, synchronous controls and ground controls) and irrespective of the age of the embryos, some of the specimen are well fixed, but we have also encountered samples that are poorly fixed. The quality of fixation impacts on both our ability to delineate the vascular morphology as well as on current and future immunohistochemical studies. Such inconsistencies in fixation had not been observed in the previous two missions, in particular not in the ground control samples. We, therefore, replaced the "holding buffer" which in our case was DEPC-treated PBS with the original fixation buffer (PBS with 4% formaldehyde), hoping to enhance the quality of fixation in the remaining samples.

By focusing on the analysis of the CAMs, we have, as yet, not examined any adrenals. We anticipate that the additional time in fixative will be beneficial for analyzing these brittle structures.

In addition to our initial preliminary observations at the time of dissection, our provisional analysis of some of the samples indicates a) retardation in the formation of the vasculature in the CAM of flight samples, and b) lack of an effect of the flight simulation conditions. Further analysis of the samples will focus on finishing the evaluation of vascular development in the CAMs by light microscopy and by immunohistochemistry, and on the assessment of the vascularization in the adrenals. Specifically, we are looking forward to obtaining the last set of ground/synchronous controls and to comparing our results to that of the other PIs in order to assess the effects of space flight on avian embryonic development.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Chekanov, V.S., Tshekanov, G.V., Rieder, M.A., Eisenstein, R., Wankowski, D.M., Schmidt, D.H., Nikolaychik, V.V., and Lelkes, P.I. Biological glue increased capillary ingrowth after cardiomyoplasty in an ischemic cardiomyoplasty model. ASAIO J., 42:M, 480-486 (1996).

Lelkes, P.I., Manolopoulos, V.G., Silverman, M., Zhang, S., Karmiol, S., and Unsworth, B.R. "On the possible role of endothelial cell heterogeneity in angiogenesis" in "Molecular, Cellular, and Clinical Aspects of Angiogenesis." Edited by: Margoudakis, M.E., Gullino, P., and Lelkes, P.I. Plenum Press, New York and London, pp 229-240, 1996.

Lelkes, P.I., Nikolaychik, V.V., Samet, M.M., Wankowski, D.M., and Chekanov, V. "Factitious angiogenesis III: How to successfully endothelialize cardiovascular bioprostheses by employing natural angiogenic mechanisms" in "Molecular, Cellular, and Clinical Aspects of Angiogenesis." Edited by: Maragoudakis, M.E. Plenum Press, New York and London, pp 229-240, 1996.

Manolopoulos, V.G., Samet, M.M., and Lelkes, P.I. Regulation of the adenylyl cyclase signaling system in various types of cultured endothelial cells. J. Cell Biochem., 57, 590-598 (1995).

Nikolaychik, V.N., Samet, M.M., and Lelkes, P.I. A new method for continual quantitation of viable cells on endothelialized polyurethanes. J. Biomat. Sci., 7, 281-288 (1996).

Nikolaychik, V.V., Wankowski, D.M., Samet, M.M., and Lelkes, P.I. I*n vitro* testing of endothelial cell monolayers under dynamic conditions inside a beating ventricular prosthesis. ASAIO J., 42:M, 487-493 (1996).

Rasouly, D., Shavit, D., Zuniga, R., Elejalde, R.B., Unsworth, B.R., Yayon, A., Lazarovici, P., and Lelkes, P.I. Staurosporine induced neurite outgrowth in neuronal hybrids (PC12EN) lacking NGF receptors. J. Biomat. Sci., 62, 356-371 (1996).

Samad, F., Bergstrom, G., Lelkes, P.I., and Amrani, D.L. Interleukin-6 regulated PAI-1 gene expression in microvascular endothelial cells. Endothelium, 3, 243-252 (1995).

Silverman, M., Manolopoulos, V.G., Unsworth, B.R., and Lelkes, P.I. Tissue factor expression is differentially modulated by cyclic mechanical strain in various human endothelial cells. Blood Coagulation & Fibrinoysis, 7, 281-288 (1996).

Thomas, G.A., Lelkes, P.I., Chick, D.M., Lu, H., Kowal, T.A., Hammond, R.L., Nakajima, H., and Stephenson, L.W. Endothelial lined skeletal muscle ventricles: Open and percutaneous techniques. J. Cardiac Surgery, 10, 245-256 (1995).

Correlation of Disconjugate Eye Torsion with the Time Course of the Space Adaptation Syndrome

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Funding:

Project Identification: 17-USA

Initial Funding Date: 7/95

FY 1996 Funding: $ 140,000

Joint Agency Participation: ESA

Solicitation:

Expiration: 6/97

Students Funded Under Research: 0

Flight Information:

Flight Assignment: Euro-Mir-95

Responsible NASA Center: ARC

Task Description:

This study proposes to document the correlation of eye torsional changes with the development and recovery over time of space motion sickness.

The hypothesis of asymmetric otolith function asserts that physiological or anatomical differences in the two sides of the bilateral gravity-sensing otolith apparatus of the inner ear may be well compensated on Earth, but when exposed to novel gravitational states, the prior compensatory stratagems may be ineffective, leading to unstable vestibular responses and causing the phenomenon of space motion sickness. To investigate this hypothesis, spontaneous eye torsion, a reflex governed by the otolith organs, was examined in the upright position during the hypo- and hypergravity of parabolic flight aboard NASA's KC-135 aircraft in thirteen former astronauts whose history of space motion sickness was revealed after data analysis had been completed (Diamond and Markham, 1991, and Markham and Diamond, in press). Results showed that astronauts who had been sick in space had significantly higher scores of disconjugate eye torsion in parabolic flight, and that their disconjugacy was consistently different in 1.8-G relative to 0-G compared to astronauts who had not been sick in space. In 1-G, there wre no differences in disconjugate eye torsion between the subjects. The results support the asymmetry hypothesis and offer a possible predictive test of space motion sickness.

Based on this work, we hypothesize that about half the subjects on the ESA Eureca flights will show a much higher level of disconjugate ocular torsion in the first few days of space flight; that these subjects will suffer space motion sickness; and that their torsional instability will return to normal in parallel with recovery from motion sickness. Some may show the inverse of this pattern on return to Earth.

We propose measuring disconjugate ocular torsion using a new but well tested infrared computer-based videooculography (VOG) technique (Clarke et al., 1991) at specified intervals before flight, during and after the likely period of getting motion sick in space, and similarly on return to Earth.

The Earth-bound measurements will be performed with the subjects in a head-upright position. Inflight measurements will require only that the subject remain stationary. In order to record images from both eyes simultaneously, the present VOG system will be augmented by one additional camera and recorder unit.

Eye torsion studies perfrormed with VOG image analysis and data reduction will take place in our laboratories at UCLA.

Three astronauts underwent preflight, inflight, and postflight testing of spontaneous ocular torsion and of ocular counterrolling (OCR) reflexes governed by the gravity-responsive otolith organs in the inner ear. One astronaut, A, had a 30-day space mission on Euromir '94 and was examined monocularly with Senso-Motoric Instruments VOG. The other two astronauts, B and C, were studied with a binocular VOG and flew an 180-day mission on Euromir '95.

In space, spontaneous eye torsion in the upright position was found to be substantially offset from baseline Earth-based recordings in all three subjects for the duration of the flights. In addition, the binocular studies showed a marked torsional disconjugacy. On return to Earth, these responses persisted for many days.

OCR in response to 30° right and left tilt was examined pre- and postflight. Compared to preflight, astronaut A showed reduced OCR immediately post-flight, increasing over the next few days. Both astronauts B and C had increased OCR postflight, which gradually approached but did not achieve the preflight values over 13 days postflight. The failure of adaptation of ocular torsion in space and slow adaptation postflight may reflect the lack of visual feedback and the open loop nature of the otolith-ocular torsion reflex.

An intrinsic part of this study is space motion sickness. We are trying to identify characteristics in eye movements, particularly rotatory eye movements during the course of space flight that may correlate with space motion sickness. We have already done significant work in predicting space motion sickness by characteristic eye movements during the course of parabolic flight; we are looking for possible similar changes during space flight.

Space motion sickness is very similar to motion sickness on Earth as found in automobiles, boats, and in aircraft. We are hopeful that information obtained on space motion sickness will have a direct impact on Earth-based motion sickness.

FY96 Publications, Presentations, and Other Accomplishments:

Anton, R., Kordower, J.H., Kane, D.J., Markham, C.H., and Bredesen, D.E. Neural transplantation of cells expressing the anti-apoptotic gene *bcl*-2. Cell Transplantation, 4(1), 49-54 (1995).

Diamond, S.G. and Markham, C.H. The effect of space missions on gravity-responsive torsional eye movements. J. Vestibular Res., (in press).

Markham, C.H. "How does the brain generate horizontal nystagmus?" in "Basic Vestibular Mechanisms." Edited by: Baloh, R.W. and Halmagyi, G.M. Oxford University Press, New York, Part 1, Ch.4, pp 48-61, 1996.

Markham, C.H. and Diamond, S.G. Further evidence to support disonjugate torsion as a predictor of space motion sickness. Aviation, Space and Environ. Med., (in press).

Markham, C.H. and Diamond, S.G. Eye torsion in space and during static tilt pre- and post-spaceflight. Proceedings of the Sixth European Symposium on Life Science Research in Space, Trondheim, Norway, June 16-20, 1996.

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

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: NRA 93-OLMSA-06

Initial Funding Date: 6/95

Expiration: 5/97

FY 1996 Funding: $60,000

Students Funded Under Research: 3

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

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.

One experiment was completed in 1996 (Mir 21/NASA-2). The biospecimens were launched on STS-76 and returned to Earth on STS-79. Although tissues of some quail embryos were not well fixed as originally expected, data from control groups and a limited amount of data from the flight experimental group have indicated that an immunohistochemical analysis can be successfully done using the brain tissues that were fixed in space.

Ten brain tissue samples have been received from the lab control groups, seven from the synchronus controls, and four from the flight groups. Among the tissues in the flight group, 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 11 tissue samples, including three tissue samples from the synchronous control group and two tissue samples 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 of 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? Tissues are currently analyzed using other antibodies, including calmodulin and different peptides. 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:

No Co-Is Assigned to this Task

Funding:

Project Identification: NAS2-14213
Solicitation: 93-OLMSA-06
Initial Funding Date: 5/95
Expiration: 4/96
FY 1996 Funding: $57,460
Students Funded Under Research: 3

Flight Information:

Flight Assignment: Euro-Mir

Responsible NASA Center: ARC

Flight Hardware Required: Incubator II Joint US/Russian

Task Description:

The fundamental question of whether complete normal embryogenesis of the Japanese quail can be accomplised in microgravity was addressed with the recently completed NASA-2 STS-76-79 flight experiment on the Russsian Space Station Mir. This study, a cooperative effort between Russian and American scientists, engineers, cosmonauts and astronauts, was designed to study morphogenesis of several organs and systems in the Japanese Quail, *Coturnix japonica*.

Preliminary data collected in October 1996 strongly indicated that successful reproductive development and embryogenesis to the time of hatching can take place in space microgravity under the specific conditions of this experiment. The quail eggs were fertilized and laid in the 1-G of Earth, held at 16°C until they were launched into space on the shuttle Atlantis (STS 76), and incubated in space microgravity of the spacelab Mir from 0 to 16 days starting incubation 6 days post lay. Subsequent histological, histochemical, and immunochemical analyses of the numerous tissues at various stages of embryonic differentiation and development is currently being conducted. The Avian Physiology Laboratory in Madison has completed the histology and analyses of the lung tissues from the 14- and 16-day-old embryos as well as some of the day-0 unincubated blastoderms, day-3 embryos, and reproductive tissues from the day-7 embryos. On the embryonic age 16 (E-16) at fixation, one bird from the flight group, two from the synchronous controls, and two from the laboratory controls had obviously started breathing. Additionally, one bird from the flight group, one from the synchronous controls, and two from the laboratory controls have yet to be examined more exactly by an avian pathologist to determine if we can more precisely define the status of these tissues. We suspect that these tissues indicated the initial steps in the initiation of breathing. Of the 14-day embryos, none had obviously established breathing. However, one embryo from the synchronous controls has also to be examined more fully by an avian pathologist, as it appeared older than the 14 days of fixation and may have also initiated a breathing mechanism. The fecundity potential as well as some of the specific morphogenic events in the developing embryo appeared normal in the unincubated eggs and the three-day-old embryos (E-3). Reproductive development in the seven-day-old embryos appears normal (samples not yet completed). Gross and microscopic abnormalities of the

embryo, E-7 #14 (that died after approximately three days of incubation) cannot be reported, as examiniation is not yet completed by the Avian Physology Laboraoty.

Our experiments are designed to foster reproduction in space microgravity. Additionally, we expect to gather substantial information on basic embryonic developmental processes. This biology will have a direct bearing on the understanding of embryogenesis and reproduction. Furthermore, we expect to gain a better interpretation about the role that Earth gravity and space microgravity have on cell and tissue migration during embryo development and differentiation. Embryonic cell migration is a primary reproductive interest. In all vertebrates, the germinal cells (future sperm and eggs) must migrate from outside the embryo to the gonads where they will proliferate and differentiate to form spermatogonia and oogonia. Some information may be forthcoming on the need for controlled turning during avian embryonic development.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Proudman, J.A. and Wentworht, B.C. Pulsatile secretion of prolactin in laying and incubating turkey hens. Domestic Animal Endocrinology, 13, 3:277-282 (1996).

Proudman, J.A., Wentworth, B.C., Ramesh, R., and Kuenzel, W.J. Prolactin secretory patterns and pituitary lactotroph changes during the reproductive cycle of turkey hens. Poultry Avian Biology Reviews, 6:297, (1996).

Wentworth, B.C., Tsai, H., Wentworth, A.L., Wong, E.A., Proudman, J.A., and El Halawani, M.E. "Primordial germ cells for genetic modification of poultry" in "Beltsville Symposia in Agricultural Research XX: Biotechnology's Role in the Genetic Improvement of Farm Animals." Edited by: Miller, R.H., Pursel, V.G., and Norman, H.D. Savoy, Illinois: American Society of Animal Science, pp. 202-227, 1996.

Pulmonary Function During Extended Exposure to Weightlessness (Euromir 95)

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Funding:

Project Identification: Euromir '95
Initial Funding Date: 7/94
FY 1996 Funding: $

Solicitation: Unknown
Expiration: 6/96
Students Funded Under Research:

Flight Information:

Flight Assignment: Euro-Mir-96
Responsible NASA Center: JSC

Task Description:

The lung is extremely sensitive to gravity, and experiments that 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 four- to six-month period in microgravity.

As an adjunct to this study, we will study the effect of raised CO_2 levels on pulmonary function in four 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.

EuroMir 95/D-2: Data collection was successfully completed in the EuroMir 95 crew, as was postflight collection. Data analysis is ongoing.

CO_2 Exposure: Data analysis is complete on this section of the project. A publication has been submitted to Aviation Space and Environmental Medicine. We are waiting for a response from reviewers.

The effect of long-term exposure to microgravity 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 humans. Such knowledge is essential in environments such as the space station where maintenance of very low CO_2 levels could become prohibitively expensive.

FY96 Publications, Presentations, and Other Accomplishments:

Dutrieu, B., Lauzon, A.M., Verbanck, S., Elliott, A.R., Prisk, G.K., West, J.B., and Paiva, M. (abstract) Reconstruction of single breath washins from multiple bolus washins under different gravity conditions. Res. Crit. Care Med., (in press).

Elliott, A.R., Prisk, G.K., Hoffmann, U., and Schoellman, C. (abstract) Response to 23 days of low level CO_2 exposure: Resting ventilation and hypercapnic response. Res. Crit. Care Med., vol. 153, A121 (1996).

Elliott, A.R., Prisk, G.K., Schoellman, C., and Hoffmann, U. Hypercapnic ventilatory response in humans before, during, and after 23 days of low level CO_2 exposure. Aviat. Space Environ. Med., (in press).

Verbanck, S., Linnarsson, D., Prisk, G.K., and Paiva, M. Specific ventilation distribution in microgravity. J. Appl. Physiol., vol. 80, 1458-1465 (1996).

Bed Rest Study (ground based for LMS)

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Funding:

Project Identification: 199-97-62-16 and 106-30-32
Solicitation: AO-OSSA-84
Initial Funding Date: 1/95
Expiration: 9/95
FY 1996 Funding: $
Students Funded Under Research: 7
Joint Agency Participation: ESA

Flight Information:

Flight Assignment: LMS, (STS-78, 1996)
Responsible NASA Center: ARC

Task Description:

LMS will be the first Spacelab mission involving human research experiments to perform a ground-based integrative science study before the flight. This study was performed in NASA's Human Research Facility at Ames Research Center, Moffett Field, California, to provide a simulation of the human life science experiments. Eight volunteers participated in the bedrest studies. 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 for the experiments. In addition, the pilot study provided the opportunity for the scientists to determine the effects of integrating individual experiments with the others planned for the mission.

The volunteers carried out a control period of 2 weeks to provide baseline measurements while ambulatory. After a simulated launch, the volunteers lay on inclined beds with their feet elevated 6° above their heads, the space flight model, for 17 days, the same length of time as the mission. This bedrest period was followed by an ambulatory recovery period for two weeks.

Subjects of the bedrest 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 determined how one experiment is affected by another experiment. In addition, results of the study directed to muscle and energy

metabolism applies to people who must be kept at bedrest on Earth or who become less active, for example, as with advancing age.

This ground-based pilot study has been completed. Dates of performance were June 30, 1995 to August 28, 1996. A major accomplishment for the mission was the development of a new workable schedule for four muscle experiments. There were also revisions made in 3 experiments planned for flight. Relative to the scientific goals of the mission, reportable data was acquired in 11 experiments. Two scientists identified interactive effects of 2 experiments on 2 others. Information conveyed by bedrest subjects to the astronauts who visited the center during the pilot study facilitated the experimental procedures during the flight. The most important question that was answered was that the protocols for the muscle experiments which involved repeated maximum contractions (exercise) did not actually prevent the loss of muscle mass and strength, i.e., muscle atrophy, the main focus of the research. The quadriceps showed an 8% decrease in strength. Circulating growth factor stimulated by exercise was depressed, and lean body mass was slightly but significantly reduced and in agreement with measurements of negative nitrogen balance acquired by a different experiment. The exercise during the muscle tests did not prevent an increase in evidence of accelerated bone resorption or the depression of body temperature. These results of the ground-based experiments are to be compared to the flight experiments.

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 bedrest. Both the pilot study and the flight experiments will yield information that enhances the understanding of reduced levels of physical activity, a problem primarily for people on Earth who are confined to bed or whose level of physical activity is reduced as a consequence of aging. The average person whose life expectancy has increased during the past 20 years, is likely to gain substantially from the knowledge acquired by this research because it clearly characterizes muscle atrophy and its extent during a brief two-week period, thus emphasizing the importance of maintaining a level of physical activity during the aging process.

FY96 Publications, Presentations, and Other Accomplishments:

Arnaud, S.B., Walker, K.R., and Hargens, A. Life and microgravity sciences Spacelab mission: Human research pilot study. NASA Tech Brief, 110395, 1-56 (April 1996).

Grichko, V.P. and Fitts, R.H. Effects of 17 days of bed rest on the enzyme and metabolite profile of the slow type I fiber. Med. Sci. Sports Exerc., (in press).

Trappe, T.A., Trappe, S.W., Costil, D.L., and Fitts, R.H. Human calf muscle function in response to 17-days of bed rest. Med. Sci. Sports Exerc., (in press).

Trappe, T.A., Trapppe, S.W., Costill, D.L., and Fitts, R.H. Time course of cardiorespiratory deconditioning with 17 days of 6° head down tilt bedrest. Med. Sci. Sports Exerc., (in press).

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).

Widrick, J.J., Sherwood, J., Bangart, J.J., Costil, D.L., and Fitts, R.H. Isotonic contractile properties of soleus muscle fibers after 17 days of bed rest. Med. Sci. Sports Exerc., (1996).

Direct Measurement of the Initial Bone Response to Spaceflight in Humans

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Funding:

Project Identification: E074
Initial Funding Date: 7/92
FY 1996 Funding: $

Solicitation: AO-OSSA-84
Expiration: 8/97
Students Funded Under Research:

Flight Information:

Flight Assignment: LMS, (STS-78, 1996)
Responsible NASA Center: JSC

Task Description:

The skeleton is constantly being broken down and rebuilt, with the processes normally occurring at equal rates. Space flight upsets this equilibrium, and the resulting imbalance between breakdown and reformation could cause lasting changes, 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 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 seven 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 in-flight 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 bedrest simulation study was 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.

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 that these tests now cost. This will add significantly to the evaluation of calcium metabolism in patients with osteoporosis and especially in other metabolic disorders of calcium.

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

Information regarding specific progress made during FY96 was not provived by the principal investigator.

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

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Funding:

Project Identification: E036
Solicitation: AO-OSSA-84
Initial Funding Date: 7/92
Expiration: 4/97
FY 1996 Funding: $
Students Funded Under Research: 11

Flight Information:

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

Responsible NASA Center: JSC

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 five muscles on the right leg and arm. Once during each of the three 24-hour tests, each payload crew member will perform movements of the right leg and arm, using the Torque Velocity Dynamometer to determine levels and patterns of EMG activity at maximum and submaximum levels of effort. Also, in this second segment of the experiment, subjects test their ability to apply pressure by compressing a hand-grip dynamometer, a device that measures grip strength. These tests will provide information on the strategies of the nervous system to regulate controlled muscular activity and on how the microgravity environment modifies these neural strategies. The results also may reveal the importance of muscle use in the learning and forgetting of motor skills and may shed light on whether unstressed muscles and their neural networks compensate appropriately so that they regain the ability to move precisely or to maintain the appropriate postures in Earth's gravity and a microgravity environment.

The effects of space flight on the fatigability of the ankle extensors (calf muscles) will be tested by having crew members perform a series of repetitive submaximal and then maximal isometric contractions. Both the torque of the ankle (force output) and the 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.

All flight and postflight experiments were completed. Data analyses are being completed and at=re in a final phase. Data will be presented at several scientific meetings during the coming year.

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

Another phase of the proposed experiments addresses a fundamentally new biological process previously undiscovered. We have found that muscle spindle receptors can stimulate or inhibit the release of growth hormone factors. Further, these receptors seem to become less efficacious with bedrest, and we hypothesize that similar effects will be caused by chronic exposure to space flight.

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

Effect of Weightlessness on Human Single Muscle Fiber Function

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Funding:

Project Identification: E920
Initial Funding Date: 7/92
FY 1996 Funding: $200,000

Solicitation: AO-OSSA-84
Expiration: 4/97
Students Funded Under Research: 6

Flight Information:

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

Responsible NASA Center: JSC

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

In FY96, we conducted the LMS (preflight, inflight, and postflight) E920 experiments. Our experiments studied four male crew members of the STS-78 Space Shuttle flight, and determined the effects of microgravity on

aerobic capacity, calf muscle function, and the cellular properties of individual slow and fast skeletal muscle fibers. A brief summary of our results are discussed here.

Though oxygen uptake (L/min) during submaximal exercise at 150 watts was unaffected by spaceflight, heart rates were elevated, on average, 10 beats/min inflight, and 18 beats/min on R+1. These values returned to the preflight values by R+5. $VO_{2\ max}$ decreased by 10% from L-15 to R+4, returning to normal by R+8. VO_2 at HR_{max} showed a continued decline throughout the flight, reaching a nadir of -15.7% during testing on F+13, which returned to the preflight level by R+5. These changes in VO_2 at HR_{max} amd VO_2^{max} appear to be associated with a decline in total body water, as reflected by the crews' loss of body weight during the adaptation to space flight and rehydration in the days following their return to Earth.

Regarding calf muscle function, the torque velocity dynamometer (TVD) was employed to assess calf muscle strength prior to flight, on flight day 2, 8, and 12, and on days 2 and 8 during recovery. Maximal isometric strength at 80, 90, and 100 degrees of ankle plantar flexion was similar during and after the flight, and the force-velocity relationship measured at six angular velocities was also unaltered by flight. Muscle fiber composition (determined by myosin ATPase staining and myosin heavy chain analysis) of the soleus (SOL) and gastrocnemius (GAST) was not altered by the 17-day space flight. Muscle fiber size of type I and II fibers of both muscles decreased by approximately 0%. The cellular studies evaluated a total of 441 single-fiber segments which were mounted between a force transducer and position motor and subjected to a series of slack length steps at maximal Ca^{2+} activation. Fibers were subsequently run on SDS polyacrylamide gels to determine myosin heavy chain (MHC) composition. The average maximal shortening velocity (V_0) of the SOL type I, GAST type I, and GAST type IIa fibers increased by 27-29% with space flight. However, only SOL-I and GAST-IIa fibers displayed significant reductions in fiber cross-sectional area (-17% and -12%, respectively). Peak force (P_0) declined in parallel with fiber atrophy for the GAST-IIa fibers but exceeded the loss of fiber area for the SOL-I fibers such that specific P_0 was reduced by 4% ($P < 0.05$). After space flight, shortening velocity at peak power output increased for the SOL type I (+29%) and GAST type I (+30%) fibers and was unchanged for the GAST-IIa fibers. In contrast, the force produced at peak power declined for SOL-I (-28%), GAST-I (-11%), and GAST-IIa fibers (-17%) due to decreases in peak force (P_0) and a/P_0 for the SOL-I fibers, a/P_0 only for the GAST-I fibers, and P_0 only for the GAST-IIa fibers. Space flight caused the absolute peak power to decrease in the SOL-I (11.0 ± 0.3 to 10.1 ± 0.3 m.N.FL.s^{-1}) and the GAST-IIa (44.5 ± 1.8 to 39.8 ± 1.9 m.N.FL.s^{-1}) fibers. The decrease in peak force and power, and the increased fiber V_0 may have resulted from a selective loss of actin filaments. This possibility is currently being evaluated. In summary, SOL-I fibers were the most and GAST-I fibers the least affected by 17 days of space flight. One possibility is that GAST-I fibers were more responsive to the countermeasure treatments employed during this flight (sub-maximal cycle ergometry) than either the SOL-I or GAST-IIa fibers.

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

FY96 Publications, Presentations, and Other Accomplishments:

Grichko, V.P. and Fitts, R.H. Effect of 17 day bedrest on the enzyme and metabolite profile of the slow type I fiber. Med. Sci. Sports Exerc., vol. 28, no. S146, (1996).

Riley, D.A., Bain, J.L.W., Thompson, J.L., Trappe, S., Costill, D., and Fitts, R.H. Ultrastructural changes in human soleus muscle fibers following chronic bedrest. Med. Sci. Sports Exerc., vol. 28, no. S146, (1996).

Romatowski, J.G., Widrick, J.J., Sherwood, J., Bangart, J.J., Costill, D.L., and Fitts, R.H. Isotonic contractile properties of soleus muscle fibers after 17 days of bedrest. Med. Sci. Sports Exerc., vol. 28, no. S146, (1996).

Trappe, S.W. ,Trappe, T.A., Costill, D.L., and Fitts, R.H. Human calf muscle function in response to 17-days of bed rest. Med. Sci. Sports Exerc., vol. 28, no. S146, (1996).

Trappe, T.A., Trappe, S.W., Costill, D.L., and Fitts, R.H. The course of cardiorespiratory deconditioning with 17 days of 6 degree head down tilt bedrest. Med. Sci. Sports Exerc., vol. 28, no. S145, (1996).

Magnetic Resonance Imaging after Exposure to Microgravity

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Funding:

Project Identification: E029
Initial Funding Date: 9/93
FY 1996 Funding: $107,000

Solicitation: AO-OSSA-84
Expiration: 9/96
Students Funded Under Research:

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

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

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

The data analysis was completed for the bedrest study at Ames Research Center which was designed to mimic the LMS flight.

All preflight and postflight data acquisition went according to plan. Additional spectroscopy scans were obtained on the crew to examine an unexpected change in the spine bone marrow. All MRI and DEXA flight data has been analyzed except for the statistical analysis.

The cross-sectional thigh and calf MRI analyses were completed, and the results were sent to Drs. Tesch and Narici.

We found a significant increase in the T2 of the cellular component in the vertebral bone marrow after flight. These changes demonstrated a time course lasting several months; the T2 of three of the four crew members remained elevated above baseline 130 days after landing.

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 1-G will cause bone and muscle loss. The loss of bone and muscle with aging occurs in both men and women, resulting in a significant public health problem. Although the exact cause of bone and muscle loss with aging is not understood, one important risk factor is disuse. Men and women become less active as they grow older, and that may play an important role in the elderly and in patients immobilized for medical reasons. In addition, muscle atrophy is an important component of many disease states as well as aging; therefore understanding the role of disuse versus other causes is important for elucidating the physiological mechanisms of muscle atrophy. The relationship of muscle atrophy to muscle performance is not well understood. The LMS flight will examine decrements in muscle performance with measurements of muscle specific atrophy.

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

FY96 Publications, Presentations, and Other Accomplishments:

Coburn, S., Thampy, K., Lane, H., Conn, P., Ziegler, P., Costill, D., Mahuren, J., Fink, W., Pearson, D., Schaltenbrand, W., Pauly, T., Townsend, D., LeBlanc, A., and Smith, S. Pyridoxic acid excretion during low vitamin B-6 intake, total fasting and bed rest. Am. J. Clin. Nutr., vol. 62, 979-983 (1995).

Curylo, L.J., Lindsey, R.W., Dogerty, B.J., and LeBlanc, A.D. Segmental variations of bone mineral density in the cervical spine. Spine, vol. 21, no. 3, 319-322 (1996).

LeBlanc, A.D. Musculoskeletal changes during long-term space flight. Second Annual Controversies in Nutrition 1996, Baylor College of Medicine, Houston, TX, August 24-25, 1996.

LeBlanc, A., Rowe, R., Evans, H., Schneider, V., and Shackelford, L. Differential muscle atrophy during bed rest. Annual meetng of Aviation, Space, and Environmental Medicine, Anaheim, CA, May 7-11, 1996.

Oganov, V.S., Schneider, V.S., Voronin, L.I., Bakulin, A.V., Murasho, L.M., Novikov, V.E., Shakelford, L., and LeBlanc, A. Space flight human bone tissue changes: The nature and possible mechanisms. Sixth European Symposium on Life Sciences Research in Space, Trondheim, Norway, June 17-21, 1996.

Smith, S., Lane, H., Nillen, J., and LeBlanc, A. Effect of real and simulated space flight on collagen crosslink excretion. Annual Meeting of the American Institute of Aeronautics and Astronautics, Houston, TX, March 5-7, 1996.

Lignin Formation and Effects of Microgravity: a New Approach

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Funding:

Project Identification:

Solicitation: 93-OLMSA-07

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $118,000

Students Funded Under Research: 5

Flight Information:

Flight Assignment: PGF/LMS, (STS-78, 7/96)

Responsible NASA Center: KSC

Flight Hardware Required: PGU

Task Description:

The focus of the plant science experiment on the Life and Microgravity Spacelab mission is to establish the effect of the microgravity environment on the ability of plants to form a reinforcement tissue known as reaction wood. On Earth, woody plants produce this distinctive reinforcement tissue when their stems are bent contrary to their normal orientation. The reaction wood formation helps restore the stem to its upright position, which contributes to the plant's survival, but 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 complement the study, enabling scientists to measure the effects of microgravity and reaction wood formation and, if possible, to define the regulatory enzymes and genes involved. The technology used for this experiment will be incorporated into future space station facilities for plant growth.

STS-78 Mission: Shuttle Columbia was launched on June 20th 1996 with a Plant Growth Unit (PGU) containing 16 Douglas firs and 4 loblolly pines, all one year old, in five Plant Growth Chambers (PGCs). After two days in orbit, members of the crew bent one half of the trees to a 45° angle. Photographs of all PGCs were taken. Ten days after launch, the trees in one PGU were harvested with the tissue being placed in a fixative bag containing gluteraldehyde/paraformaldehyde, where they were kept until return on Earth. Again, photographs were taken. Thirteen days after launch the same manipulations were repeated with another PGC. Upon return to

Earth on on July 7, 1996, the trees in the remaining 3 PGCs were harvested and again the appropriate tissue was placed in the fixative solution. The same experiment was also conducted at KSC as a ground control. Upon return to Washington State University, the tissues were analyzed for reaction wood formation using electron microscopy.

Metabolic flux of phenylpropanoid monomers into lignin has been investigated. When treated with 8% sucrose and 20 mM KI, *Pinus taeda* cell culture accumulate both *p*-coumaryl and coniferyl alcohols in the culture medium as well as the lignans pinoresinol, dehydrodiconiferyl alcohol and guaiacylglycerol 8-*O*-coniferyl alcohol ether. Methodolgy was developed to examine the changes in metabolite profile of subcellular components, which has led to defining the rate-limiting steps in the pathway to lignin.

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

FY96 Publications, Presentations, and Other Accomplishments:

Bernards, M.A. and Lewis, N.G. Suberin: An hydroxycinnamic acid-derived polymer. Polyphenols Actualities, 14, 4-6 (1995).

Davin, L.B. and Lewis, N.G. Lignin and lignan biochemical pathways in plants: An unprecedented discovery in phenolic coupling. An. Acad. Bras. Ci., 67 (Supl. 3), 363-378 (1996).

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Kostova-Dinkova, A.T., Gang, D.R., Davin, L.B., and Lewis, N.G. The lignin/lignan question in heartwood formation. Keystone Symposium. The Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology, Tamarron, CO, March 1996.

Lewis, N.G. Contrasts in lignin/lignan biosynthesis and function. Meeting of the German Botanical Society, Dusseldorf, Germany, August 1996.

Lewis, N.G., Dinkova-Kostova, A.T., Gang, D.R., and Davin, L.B. Purification, cloning and overexpression of pinoresinol/lariciresinol reductase. 211th American Chemical Society National Meeting, New Orleans, LA, March 1996.

Lewis, N.G. and Davin, L.B. Lignans in plant defense: Formation and properties. International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 1995,

Lewis, N.G. and Davin, L.B. Phenolic coupling in lignin and lignan formation. 211th American Chemical Society National Meeting, New Orleans, LA, March 1996.

Lewis, N.G., van Heerden, P., Bedgar, D.L., and Davin, L.B. Podophyllum and sesame lignan synthesis *in vivo*. International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 1995.

Razal, R.A., Ellis, S., Lewis, N.G., and Towers, G.H.N. Nitrogen recycling during phenylpropanoid metabolism. Phytochem., 41, 31-35 (1996).

Towers, G.H.N., Singh, S., van Heerden, P., and Lewis, N.G. Integrating nitrogen and phenylpropanoid pathways: The evolutionary transition of plants to a dryland habitat. 211th American Chemical Society National Meeting, New Orleans, LA, March 1996.

Van Heerden, P., Towers., G.H.N., and Lewis, N.G. Nitrogen metabolism in lignifying *Pinus taeda* cell cultures. J. Biol. Chem., 271, 12350-12355 (1996).

Human Sleep, Circadian Rhythms and Performance in Space

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Funding:

Project Identification: E948
Initial Funding Date: 8/90
FY 1996 Funding: $280,000

Solicitation: NRA
Expiration: 8/97
Students Funded Under Research:

Flight Information:

Flight Assignment: LMS, (STS-78, 1996)
Responsible NASA Center: JSC

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 six minutes. Circadian rhythms also will be evaluated by measuring urine electrolyte and hormone concentrations at each voiding, by mood and activation testing every two hours during the wake cycle, and by performance testing before each meal. Crew members 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 1996 saw our experiment flown on STS-78. Two mission specialists and two payload specialists completed two 72-h measurement blocks (FD 3-6 and 13-16) during the 17-day mission. The block ran from start of shift

to start of shift (hence impacting four mission days while covering 72h). In addition, all subjects completed preflight and postflight BDC.

Good data were obtained for most measures. Acceptable body temperature rhythms were obtained from all four astronauts, though some BDC data were lost. Similarly, sleep recordings were available from all four astronauts in preflight BDC and early-flight conditions and from three astronauts in late-flight and post-flight BDC conditions. All PGSC data (mood, alertness, performance, sleep diary, end-of-shift questionnaire) from all four astronauts was good. A 180-day preliminary report was submitted January 7, 1997 and a manuscript for peer review is currently in preparation.

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 are often impaired. Study of sleep, circadian rhythms, and performance in space allows us to understand what happens to people when they are removed from most of the time cues on Earth. Findings from our experiment will thus help us to understand the actions of zeitgebers on the human circadian system, and will help us in providing useful coping strategies to night workers and those suffering from jet-lag.

FY96 Publications, Presentations, and Other Accomplishments:

Monk, T.H., Buysse, D.J., Reynolds III, C.F., Kupfer, D.J., and Houck, P.R. Subjective alertness rhythms in elderly people. Exp. Gerontology, vol. 11, no. 3, 268-276 (1996).

Monk, T.H., Buysse, D.J., Reynolds III, C.F., Kupfer, D.J., and Houck, P.R. Circadian temperature rhythms of older people. Exp. Gerontology, vol. 30, no. 5, 455-474 (1995).

Canal and Otolith Integration Studies (COIS)

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Funding:

Project Identification:

Solicitation: 93-OLMSA-07

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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; and (4) in the immediate postflight period, responses will reflect the nature and degree of the inflight adaptation.

Based on these four points, our inclusive hypothesis predicts that during space flight there will be a modification of the normal synergy that exists to coordinate canal, otolith, proprioceptive, and other sensory input. The first part of this investigation was completed during the STS-42 mission Microgravity Vestibular Investigations

(MVI) using passive rotational stimuli. The goal of this research is to complete the MVI scientific objectives as they were originally proposed related to visual-vestibular contributions to active goal-directed spatial orientation tasks.

Data Collection

Pre- and postflight data were collected on four subjects. Three preflight (80, 45, and L-15 days before launch) and five postflight (0, 1, 4, and 8 days after landing) data collections were performed.

Status of Data Analysis

- The pre- and postflight data pre-processing has been completed. Effort is now concentrated on quantitative analysis of all data.
- The in-flight data has been stripped from the tapes and all pre-processing of the data has been completed. Effort is now concentrated on quantitative analysis of all data.

Preliminary Research Findings

- Postflight testing showed what appears to be significant changes in the pursuit eye movement system.
- Initial inspection of the data from the optokinetic eye movement experiment showed strong interactions with head position and gravity during the postflight tests. Hypothesized, but never observed before, was a trend to switch eye movements from the horizontal plane (side-to-side eye movements) for eye movements in the vertical lane (up and down eye movements) when the while body was tilted from the vertical position.
- It was also interesting to note that the crew members during postflight testing consistently over-estimated their angle of tilt.
- Due to the complexity of the analysis, no preliminary conclusions could be drawn from the sinusoidal head oscillations portion of the experiment. Preliminary analysis is now complete for all subjects on all phases of the flight.

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

Microgravity Effects on Standardized Cognitive Performance Measures

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Funding:

Project Identification: E963
Initial Funding Date: 11/89
FY 1996 Funding: $265,500
Joint Agency Participation: DoD

Solicitation: 89-OSSA-13
Expiration: 9/97
Students Funded Under Research: 5

Flight Information:

Flight Assignment: LMS, (STS-78)
Responsible NASA Center: JSC
Flight Hardware Required: PAWS (laptop computer, task ball)

Task Description:

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

With the successful conclusion of the Life and Microgravity (LMS) preflight and postflight baseline data-collection periods and in-orbit evaluations, we now have objective, broad-based cognitive performance measures on seven astronauts, three from the International Microgravity Laboratory (IML-2) mission and four additional astronauts from the LMS mission. These data will allow us to assess the impact of two 15 to 17-day periods in orbit for any changes in memory, attention, mathematical calculations, and visual-motor skills. On LMS, each of four astronauts completed 37 sessions of a 20-minute battery of six cognitive performance tests and two subjective scales on a laptop computer. Twenty-four of the sessions were preflight, nine sessions were in-orbit, and four sessions were post-flight. A total of 1183 data sets were recorded from the astronauts for the

experiment. The data from one subject, on a single test, was not recorded in-orbit. All other data have been transcribed and reduced for analysis. Graphs of the summarized raw data indicate that subjective fatigue increases toward the end of the mission, and this condition may lead to performance decrements. A complete analysis of these results will tell us if cognitive functioning is unaffected or if performance degrades in the weightless environment.

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

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

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

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

FY96 Publications, Presentations, and Other Accomplishments:

Schiflett, S.G., Eddy, D.R., Schlegel, R.E., French, J., and Shehab, R.L. Performance assessment workstation (PAWS): Microgravity effects on standardized cognitive performance measures. Final report for International Microgravity Laboratory (IML-2) mission, Brooks AFB, TX, November (1995).

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

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Funding:

Project Identification: E871
Initial Funding Date: 1/96
FY 1996 Funding: $374,579

Solicitation: AO-OSSA-84
Expiration: 12/96
Students Funded Under Research:

Flight Information:

Flight Assignment: LMS, (STS-78, 1996)
Responsible NASA Center: JSC

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 (180) 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 CO_2 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 two consecutive six-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 inflight data against the combination of the preflight and matched bedrest data will indicate whether the energy costs of living and working in space are greater or less than those on Earth for comparable activity.

During FY 1996, most of the analyses from the ground control bedrest study were completed, and the flight experiment was carried out. To date, the principal findings from the bedrest study have been the comparisons of the bedrest data against the data from SLS1 and SLS2. Data are means ± SE with the number of subjects in ().

Nitrogen retention based on excretion of nitrogen in the urine was reduced during both bedrest from 22 ± 1 (7) to 1 ± 5 (7) mg N. $kg^{-1}.d^{-1}$, ($p<0.05$) for bedrest and space flight (from 57 ± 9 (9) to 19 ± 3 (9) mg N. $kg^{-1}.d^{-1}$, ($p<0.05$). 3-Methylhistidine (3-MeH) excretion was unchanged with either bed rest, (pre-bedrest 5.30 ± 0.29 (7) vs. bedrest 5.71 ± 0.30 (7) mmol 3-MeH. $kg^{-1}.d^{-1}$, p=ns) or space flight, (preflight 4.98 ± 0.37 (9) vs 4.59 ± 0.39 (9) mmol 3-MeH. $kg^{-1}.d^{-1}$ inflight p=ns). Conclusions: (i) 3-MeH excretion was unaffected by space flight on the shuttle or bedrest plus exercise. (ii) Since protein breakdown (elevated 3-MeH) was increased on Skylab but not on the shuttle, it follows that muscle protein breakdown is not an inevitable consequence of space flight.

The flight experiment was successfully completed during the time frame June to August of 1996. Yield of samples (saliva and urine) was about 90% of planned. This should provide an excellent data set and will enable us to meet the objectives of the experiment. Analyses are still in progress.

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
Initial Funding Date: 7/92
FY 1996 Funding: $483,000

Solicitation: AO-OSSA-84
Expiration: 6/97
Students Funded Under Research: 3

Flight Information:

Flight Assignment: LMS, (STS-78, 1996)
Responsible NASA Center: JSC
Flight Hardware Required: ALFE/GASMAP/LSLE Micro-2

Task Description:

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

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

LMS flew in mid 1996. The ALFE package was used as planned for pre- and post-exercise data collection four times throughput the mission. In addition, three extra pre-exercise sessions on the payload crew were performed,

and the three orbiter crew members performed pre-exercise sessions on an as-available basis. Two inflight anomalies required the performance on inflight maintenance procedures. An oxygen tank leak pre-launch necessitated the routing of a hose and mask from the middeck. This recovered the post-exercise pulmonary blood flow measurements, but not the distribution of ventilation studies. Torso extension in the crew necessitated the lengthening of the RIP suit shoulder straps in some crew. This resolved the fit problems in all payload crew members affected. The remainder of the inflight data collection proceeded without difficulty. Complete pre- and post-flight data sets collected on all seven crew. Data analysis is ongoing.

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

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

Development of the Fish Medaka *in Microgravity*

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Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1996 Funding: $198,989

Solicitation: Unsolicited

Expiration: 12/97

Students Funded Under Research: 5

Flight Information:

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

Responsible NASA Center: ARC

Task Description:

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

Flight Studies Overview: FY1996 included a flight experiment on LMS on June 20, 1996. Thirty-six *medaka* embryos were flown on a modified STL-B hardware system. Digital images and real-time video sequences were taken of the flight, synchronous ground, and non-synchronous ground embryos under identical conditions except for the flight environment (acceleration into orbit and microgravity). In addition, studies have been performed on the use of reduced temperatures to study early embryogenesis, on developing alternative fixation protocols, on gene expression in *medaka* embryos and on the effects of retinoic acid on embryogenesis in developing *medaka*. Embryos were intended to be fixed at intervals of Orbit plus 24, 48, 72, 96, 142, and 166 hours. A hardware malfunction led to the Orbit plus 142- and 166-hour embryos not being fixed until the shuttle landed back on Earth. All of the embryos, both flight and control, have been embedded, and many have been sectioned. Analysis of the video and digital data are underway.

Use of Reduced Temperatures in Studies of Early Embryogenesis in the Microgravity Environment: 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 was 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. In STS-78, temperatures were held at 12°C during loading of the embryos and in the STL-B hardware unit until orbit was achieved. Once in orbit, the system raised the temperature in the chambers to 17.5°C, which allowed development to proceed at a faster rate. The temperature was monitored throughout the flight and held to within 1 degree.

One important consequence of the previous flight experiments was the preliminary observation that flight embryos might develop at slightly different rates than do the ground controls. Analysis of developmental rate differences in fish embryos will be highly dependent on controlling and monitoring the temperature during both the flight and control samples. As noted above, the STL-B can maintain temperatures with a one degree centigrade accuracy. Therefore, in order to study the effects that microgravity might have on rates of development, it is important to understand the effects of small temperature changes, up or down, on the developmental rates between specific stages of embryonic development. We have done extensive studies on the effects of temperatures at 14°C, 15°C, and 16°C, concentrating on the period of development encompassing gastrulation. Interestingly, embryos raised at 14°C appear to develop faster during the initiation of movements at gastrulation than do embryos raised at either 15° or 16°C. By mid-gastrulation, the developmental rates are equivalent between embryos raised at these three temperatures. Time lapse video analysis is currently being done to determine the cause and mechanisms involved in these differences during early gastrulation.

Studies on Fixation Protocols: Our preliminary histological evaluations from STS 70 suggested that 4% paraformaldehyde in the space flight condition yielded adequate but not optimal histological observations. While 4% paraformaldehyde is the fixative of choice for use in fixing tissues for subsequent *in situ* hybridixation analysis, it does not appear to be the best fixative for morphological integrity of *medaka* embryos, particularly when fixed through the chorion. Since Bouin's fixative has been used by one of us (C. Phillips) in an extensive series of studies and was found to yield both optimal morphological preservation and adequate detection of at least certain cellular proteins by immunohistochemistry, this was the fixative of choice for STS-78.

Morphological Analyses: Comparison of developmental morphology between embryos raised in microgravity with those raised on Earth requires a very detailed analysis of the three-dimensional morphology of the embryo as it develops through time. It is particularly difficult to compare serial sections from different embryos which can not be precisely oriented prior to sectioning. Therefore, we have developed a computer program which reconstructs a three-dimensional "embryo" from the serial sections and animates its development through several developmental stages. We have recently used such an approach in studies on frog embryos and are now appliying this to the fish embryos.

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

Effects of Retinoic Acid on Developing *Medaka* Embryos: Retinoic acid had been shown to be an important regulator of vertebrate development, in both the fish and mouse. In concurrent studies in our lab, we have shown that some Hox genes in the mouse, particularly Hoxa-4 (Alan Packer and D. J. Wolgemuth, unpublished observations), are regulated at least in part by the administration of all-trans retinoic to pregnant females. Although no retinoic acid binding element has yet been identified in the upstream region of *medaka* Hoxa-4, we wanted to know if this mode of regulation of the expression of this gene in mouse could be conserved through

other species. *Medaka* embryos at various stages prior or at the begining of Hoxa-4 expression (see above) were treated with all-trans retinoic acid for two hours in the dark, Embryos treated with 1uM did not show any specific phenotype, although none of them were allowed to develop further than stage 27. Embryos treated with 10 uM retinoic acid died at stage 20-21, with a typical curly tail, proving that the normal pattern of body development was strongly affected at that concentration. Whole-mount analysis of treated embryos showed that, at both concentrations, Hoxa-4 expression in the neural tube was shifted more anteriorly, and that the amplitude of the shift could be affected by the concentration of retinoic acid. Interestingly, the shift observed at stage 25 exhibited two bands of expression, more anterior than the normal limit of Hoxa-4 expression and very similar to what we have observed in the mouse (A. Packer and D.J. Wolgemuth, unpublished observations).

Video Analysis of *Medaka* Development in Control and Flight Environments: *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 three separate occasions. The first, STS-59, was considered a hardware flight test and was not supported by NASA. *Medaka* embryos were flown on this mission, and all systems checked out in terms of biocompatibility. The second and third flights, STS-70 and -78, provided the opportunity for a series of video observations and fixation of embryos for subsequent histological examinations on the effects microgravity on the development of the *medaka* at various stages. These analyses are currently underway. Video data from the flight experiments have provided, for example, 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 in the process of a detailed analysis of this and other phenomena.

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

It is commonly believed that some species make use of Earth's gravitational field as a positioning cue during early embryogenesis. It is our intention to determine: 1) if embryos can develop normally without such cues; 2) to determine if some stages of early embryogenesis are affected; and 3) at which point the affected stages regulate back to producing normal embryos. Through study of animal development, the ultimate objective is to understand the potential effects on human embryonic development and determine the risks on human reproduction induced by microgravity stress during long lasting space flights. Our studies address aspects of fundamental biology concerning vertebrate development at the molecular, cellular, and physiological levels in the environment of microgravity. Since the studies are conducted on vertebrates, the results can be more readily extrapolated to other mammalian models, particularly humans. It is commonly accepted that normal development rests largely on the embryo's ability to maintain a highly coordinated program, temporally and spatially, of morphogenetic events. Interference with the normal program of development, that is, an alteration in the carefully orchestrated cell-cell interaction, cellular migration, and cell death that should be occurring during normal embryogenesis, could result in development abnormalities at morphological, physiological, behavioral, and other levels. These abnormalities could be evident immediately or might not be apparent until later in life. Animals that develop, are born, reared, and reproduce in space may exhibit profound or more subtle morphological, physiological, behavioral, and other changes, that our experiments should help to evidence. In addition, our studies have the additional long-term potential of providing a vertebrate model for studies on the effects of others aspects of the flight environment, including radiation, as *Medaka* fish has been used as a vertebrate (but non-mammalian) test system for studying radiation-induced mutagenesis.

FY96 Publications, Presentations, and Other Accomplishments:

Phillips, C.R., Moore, J., Whalon, B., and Danilchik, M. Gravitational affects on the rearrangement of cytoplasmic components during axial formation in Amphibian development. Adv. Space Res., 17, 225-235 (1996).

Role of Corticosteroids in Bone Loss during Space Flight

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Funding:

Project Identification:

Initial Funding Date: 9/93

FY 1996 Funding: $101,848

Solicitation: AO-OSSA-84

Expiration: 8/97

Students Funded Under Research: 1

Flight Information:

Flight Assignment: LMS (STS-78, 1996)

Responsible NASA Center: ARC

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 LMS mission (STS-78) landed on schedule at KSC on July 7, 1996 after 17 days of orbital flight. After their body weights were recorded, twelve flight rats housed in two AEMs were necropsied that day followed by necropsies of 24 ground-based control rats on July 9. Blood samples, adrenal glands, thymus glands, and various bones were collected during these necropsy procedures. Both adrenalectomized (ADX) and intact flight rats gained weight normally during the experimental period. The intact flight rats exhibited a statistically significant increase in adrenal gland weight compared to ground-based control rats, which is consistent with corticosteroid excess in the former animals. Serum corticosterone levels have been measured in all rats by radioimmunoassay methods. The ADX flight and ground-based control rats had mean serum values of 60-60 ng/ml, which indicated that the corticosteroid pellets implanted subcutaneously in these animals successfully delivered physiologic levels of the hormone to the systemic circulation. In contrast, the intact flight rats had a mean serum value of nearly 500 ng/ml of corticosterone. However, this markedly elevated serum level of the hormone is probably indicative of the stress of re-entry and post-flight handling rather than a response to space flight alone. In any case, the serum data indicate that the corticosteroid pellets implanted in ADX rats were successful in delivering physiologic levels of the hormones to the systemic circulation. Furthermore, the adrenal hypertrophy detected in intact flight rats provides strong evidence for the occurrence of corticosteroid excess during space flight in these animals. Therefore, the flight experiment was flawlessly implemented.

Extensive histomorphometric anlyses have been performed on several bones from each rat. The results indicate that space flight has minimal effects on the bones of rapidly growing rats. Flight rats, whether intact or ADX with corticosteroid supplementation, had normal levels of cancellous bone mass, bone formation, and bone resorption when compared to ground-based control rats. Fulfilment of our experimental objective (i.e., to test the hypothesis that corticosteroids contribute to bone loss during space flight) was dependent on the occurrence of bone changes in intact flight rats. The failure of such bone changes to develop has, therefore, prevented us from adequately testing this hypothesis.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Li, M., Shen, Y., Halloran, B.P., Baumann, B.D., Miller, K., and Wronski, T.J. Skeletal response to corticosteroid deficiency and excess in growing male rats. Bone, 19, 81-88 (1996).

Expression of Contractile Proteins in Microgravity

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Funding:

Project Identification:

Initial Funding Date: 7/95

FY 1996 Funding: $67,867

Solicitation: 93-OLMSA-06

Expiration: 6/97

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NASA-Mir-1B, SLM-1A

Responsible NASA Center: ARC

Task Description:

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

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

RNA was isolated from E16 control hearts. We were able to isolate, using a modification of our RNA purification from fixed tissue protocol, sufficient RNA from a small portion of a heart to perform our reverse transcriptase-polymerase chain reaction (RT-PCR) experiments. To demonstrate that our present method will successfully yield the anticipated RT-PCR products, we used four different oligonucleotide primers. This allowed us to amplify a sequence from the 5¢ region of the cardiac troponin T transcript that includes a sequence

whose incorporation in cardiac troponin T cDNA is developmentally regulated and a sequence found in the center of the cardiac troponin T transcript. The latter contains no sequences that are alternately spliced so that only a single product is anticipated, while the amplified 5¢ region of the molecule should yield two products. A single band approximately 250 nt in length was appropriately generated by the forward and reverse primers, AACCGCCTGGCTGAAGAGCGC; CAGCTGATCTTCATTCAGGTG, respectively. Using the forward and reverse primers, CTCGAATCTAGAATGTCTGACCTGGAAGA and GGGCATGAAGGGCCTGGGCTT, to amplify the 5¢ region, two products of the appropriate size were obtained. These results demonstrate that a small portion of an E16 embryonic heart is sufficient for us to obtain enough RNA to perform multiple RT-PCR reactions. These results also demonstrate that the oligonucleotide primers generate only a single or doublet, as is appropriate for the region of the molecule being amplified, and that the size of the products are appropriate. To allow quantitation of the relative amounts of the products from one heart to another and from one embryonic age to another, we are presently applying a modified silver stain protocol for identifying RT-PCR products in polyacrylamide gels. We are also developing and applying appropriate primers for our investigating the regulated expression of cardiac and slow skeletal muscle troponin I in the embryonic heart. After establishing this technique, we will carry out the proposed experiments to test in the embryonic heart whether microgavity affects the regulation of transcription of thin filament proteins, essentially for cardiac contraction.

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) the flight group, eggs laid on earth and fixed in space at gestational ages of 7, 10, 14, and 17 days; 2) the time-delayed synchronous animal group (a control group sacrificed to mirror the flight group); and 3) laboratory control eggs. 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 humans and birds, the results of this study will be relevant to human development and function in space.

FY96 Publications, Presentations, and Other Accomplishments:

Anderson, P.A. Temporary Member, Cardiovascular A, NIH Study Section, 1996.

Anderson, P.A. Pediatric Ground Rounds, Transposition of the Great Vessels in Neonate. Duke University Medical Center, Durham, NC, August 1996.

Anderson, P.A. The Neonate with Congenital Heart Disease. Durham Regional Hospital, Durham, NC, March 1996.

Anderson, P.A.W. Cardiac troponin T isoform expression correlates with pathophysiological descriptors in patients who underwent corrective surgery for congenital heart disease. Circulation, 94, 472-476 (1996).

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 1996 Funding: $ 150,000

Students Funded Under Research: 4

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Task Description:

Exposure of crew, equipment, and experiments to environmental radiation during extended space missions such as space station habitation and planetary exploration poses complex scientific and technological problems which need to be resolved before accurate prediction of accumulated doses and adequate radiation protection can be achieved. The development of environmental cosmic ray and trapped radiation models and of computer codes for propagation of radiation through matter is essential to the space radiation protection effort, so that dose rates in spacecraft can be predicted from orbit, date and duration of flight and the physical attributes of the spacecraft. Detailed experimental mapping of the space radiation environment is necessary for comparisons with and rectification of the predictive models and codes. The NASA-Mir Program provides an opportunity to extend the present database of U.S. measurements of the space radiation environment to the 51.6 degree inclination of the Mir space station orbit. Since the U.S./International space station is likely to occupy a similar orbit, radiation measurements on Mir can also be used for extrapolation of dose rates to the U.S. space station environment. Intercomparisons of U.S. and Russian space radiation measurements from both passive and active detectors are needed to determine the equivalence between different instruments and techniques. Project 1-Internal is a three-year program to perform a systematic series of passive radiation-detector exposures on Mir. Concurrent measurements of absorbed dose, LET spectra (LET >5 keV/micron in water) will be made inside Mir. In Project 2-External, depth dependence of absorbed dose and LET spectra will be measured under thin shielding with dosimeter stacks on the external surface of the Mir. The internal measurements will be compared with measurements from Russian dosimeters and the JSC-TEPC active microdosimeter, exposed in the same location, and all measurements will be compared with calculations made for similar conditions by the currently available space environment and radiation transport models. The combination of internal and external measurements will yield detailed information on shielding effectiveness in the 51.6 degree orbit. The systematic series of measurements made during the approach of solar minimum (Sept. 1997), will measure solar cycle effects on environmental radiation levels and include the maximum doses of galactic cosmic rays for this cycle.

Six sets of passive radiation detectors have been deployed throughout the interior of the Mir Station during each of the misions of the NASA/Mir Science Program. Results of dose-rate measurements using TLDs are now available for the first two missions. On NASA-2/Mir 21 (March 22 - September 26, 1996) dose rates inside the Mir Base Block were found to range from 288 ± 9 to 407 ±13 microGy/day and a dose rate of 271 ± 9 microGy/day was measured inside the Kvant 2 module. On NASA-3/Mir-22 (September 16, 1996 - January 22, 1997) measured dose rates ranging from 273 ± 8 to 378 ±12 microGy/day inside the Mir Base Block, and a dose rate of 265 ± 8 microGy/day was measured inside the Kvant 2 module. Preliminary LET flux spectra from the NASA-2/Mir-21 mission measured inside the Mir Base Block is presented. Comparisons will be made between the results of recent dose rate and LET spectra measurements aboard Mir Station and both model calculations and measurements using active and passive detectors. Currently, measurements are underway on the external surface of Mir Station to provide dose rates and LET spectra under low (< 0.5 g/cm^2) shielding.

Other activities of this research include:

- Measure mission dose equivalent rates and LET spectra using passive dosimeters on NASA-2 and -3.

- Map internal radiation environment of Mir using 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.

Adaptive Changes in Cardiovascular Control at μG

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Funding:

Project Identification: E712
Initial Funding Date: 1/95
FY 1996 Funding: $426,164

Solicitation: 94-OLMSA-01
Expiration: 11/98
Students Funded Under Research:

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: Chibis, Transcranial Doppler, CBPD, GASMAP, ECG System, etc.

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 1-G. The primary hypothesis is that adaptation to the unique environment of microgravity minimizes the dynamic demands on the cardiovascular neural control. The level of physical activity is decreased, and no postural adjustments are required. This regulatory environment is likely to degrade important control mechanisms.

The experimental design represents an integrated approach to the testing of this primary hypothesis. The following questions will be answered: 1) Does efferent sympathetic nerve activity increase appropriately in response to baroreflex and non-baroreflex-mediated stimuli after space flight? 2) Can integrated clinical tests of autonomic function detect functional impairment and can they be used to characterize the time course of adaptation to microgravity? 3) Does regulation of the cerebral circulation change in parallel with or independent of the regulation of the systemic circulation? 4) Can advanced mathematical models of neural control including both linear and non-linear dynamics be developed to gain insight into the integration among neurocirculatory variables and control mechanisms? A series of well-defined physiological stimuli has been defined, including lower body negative pressure, a cold pressor test, isometric exercise, Valsalva, and controlled breathing. Responses are characterized by multiple measurements including heart rate, continuous finger arterial pressure

and direct recording of muscle sympathetic nerve traffic. The U.S. Mir experiments will enable us to extend the Neurolab observations to flights of long duration.

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

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 has been developed in collaboration with JSC. A parallel experiment has been implemented for the German flight Mir '96 with Dr. Friedhelm Baisch, DLR, Cologne, as principal investigator, and Dwain Eckberg et. al. of the Medical College of Virginia and the University of Texas Southwestern Medical Center group as Co-Is.

The protocols have been approved by the JSC IRB. The integrated payload requirements document (IPRD) has been accepted. Procedures have been completed and a Mir-24 familiarization session has been held.

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

FY96 Publications, Presentations, and Other Accomplishments:

Baisch, F.J., Blomqvist, C.G., Eckberg, D.L., and Robertson, D. "Cardiovascular regulation in microgravity and after return to 1g involvement of volume, pressure, and force changes in the modulation of automatic function" in "Research Program of the German Space Mission Mir '97." Edited by: Sahm, P.R. DLR/Cologne, pp 77-82, 1996.

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Blomqvist, G.C., Levine, B.D., Lane, L.D., and Buckey, J.C. "Space medicine and physiology" in "Atlas of Cardiology." Edited by: Braunwald, E., et.al. 1996.

Buckey, J.C. Jr., Gaffney, F.A., Lane, L.D., Levine, B.D., Watenpaugh, D.E., Wright, S.J., Yancy, C.W. Jr., Meyer, D.M., and Blomqvist, C.G. Central venous pressure in space. J. Appl. Physiol., vol. 81, no. 1, 19-25 (1996).

Buckey, J.C. Jr., Lane, L.D., Levine, B.D., Watenpaugh, D.E., Wright, S.J., Moore, W.E., Gaffney, F.A., and Blomqvist, C.G. Orthoststic intolerance in spaceflight. J. Appl. Physiol., vol. 81, no. 1, 7-18 (1996).

Levine, B.D. "Critical discuaaion of research issues in mechanisms of cardiovascular adaptation to actual and simulated microgravity" in "Medicine and Science in Sports and Exercise, Supplement." Edited by: Blomqvist, C.G., Raven, P.B., and White, R.J. vol. 28, pp S1-S112, 1996.

Levine, B.D., Lane, L.D., Watenpaugh, D.E., Gaffney, F.A., Buckey, J.C., and Blomqvist, G.C. Maximal exercise performance after adaptation to microgravity. J. Appl. Physiol., vol. 81, no. 2, 686-694 (1996).

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 1996 Funding: $275,000
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Task Description:

In the microgravity environment of space flight, the relationship between sensory input and motor output is altered. During prolonged missions, neural adaptive processes come into play recalibrating the central nervous system (CNS) to permit new sensory-motor strategies to emerge in the novel sensory environment of microgravity. However, the adaptive state achieved on orbit is inappropriate for a 1-G environment leading to postural and gait instabilities and 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; and 2) Define the pre- and postflight energy transfer between the lower limbs and the head, lower limb kinematics, and muscle activation patterns during overground locomotion.

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

Summary of Progress

1) Integration of video motion analysis system with force plate, accelerometer, electromyographic (EMG), and EOG data acquisition systems (see below for details).
2) Set-up and test of hardware in Star City, Russia.
3) Mir-21 pre and postflight data were collected on two subjects. Three preflight (120, 45, and 10 days before launch) and four postflight (1, 3, 7, and 180 days after landing) data collections were performed. One preflight sessions was collected at the Johnson Space Center (JSC). All other data collections occurred at the Gagarin Cosmonaut Training Center (GCTC), Star City, Russia.
4) NASA-3 pre- and postflight data were collected on one subject. Three preflight (120, 45, and 10 days before launch) and five postflight (4 hours, 1, 5, 7, and 12 days after landing) data collections were performed. One preflight session was collected at GCTC; all other data collections occurred at JSC.
5) Mir-22 pre- and postflight data were collected on two subjects. Three preflight (120, 45, and 10 days before launch) and four postflight (1, 3, 7, and 11 days after landing) data collections were performed. One preflight session was collected at JSC; all other data collections occurred at the GCTC.
6) NASA-4 preflight data were collected on one subject. Three preflight (120, 45, and 10 days before launch) sessions were performed. Postflight session will be collected immediately after the STS-84 landing scheduled for May 25, 1997.

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 EMG from the neck, trunk, and right lower limb, 4) vertical and horizontal eye movements using DC-EOG, 5) heel strike and toe off using footswitches, 6) ground-reaction forces during overground locomotion, and 7) dynamic visual acuity measures during treadmill walking. The following equipment is integrated to form the data acquisition system: 1) a six camera, high resolution video motion system (Motion Analysis Corp., Santa Rosa, 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 footswitches (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.

Data Analysis Summary: (as of March 97)
Mir-21: All preflight and two postflight video data sessions have been processed.
Mir-22: All preflight and one postflight video data sessions have been processed:
NASA-3: All preflight and two postflight video data sessions have been processed.
NASA-4: One preflight session has been processed.

Data Analysis Process

1. Video Data Reduction
• Tracking: calculating 3-dimensional trajectories of each body-fixed marker using the computer-based algorithms and camera calibration procedures.
• File Transfer: converting data to analyzable MATLAB format and transferring the 3-D trajectory output files and electrooculography and footswitch data to a computer network. This includes renaming trajectory variables and synchronizing EOG and footswitch analog data with trajectory traces.

These are preliminary steps that are required before further data analyses can be performed. We are now in the process of reducing the remainder of the postflight data using the steps described above.

2. Video Kinematic Analysis
X, Y, Z movement trajectories are obtained from markers placed on the head, torso, and legs, and the following parameters will be derived:
• Coherence between trunk movement and compensatory head movements.
• Amplitude of the predominant frequency of pitch, yaw, and roll head movements obtained through Fourier Analysis.
• Phase plane analysis of lower limb kinematics.

3. EMG Analysis Of Leg Muscle Activation Patterns

EMG was collected from the following muscles: left and right sternocleidomastoid, semispinalis capitus, left erector spinae, right tibialis anterior, right gastrocnemius, right rectus femoris, and right biceps femoris. The following parameters will be derived:
• Pearson R correlations between normalized pre- and postflight waveforms.
• Coefficient of variation (i.e. standard deviation divided by the mean amplitude) around both heel strike and toe-off phases of locomotion.

4. Eye Movement Analysis

EEOG was used to collect compensatory horizontal and vertical eye movement during locomotion. The following parameters will be derived:
• Coherence between trunk movement and compensatory eye movements.
• Phase relationships between eye, head and trunk movements.

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, and postural and locomotor control. These investigations are aimed at determining the magnitude and time constants of adaptation to microgravity and readaptation to Earth gravity as a function of space flight mission duration.

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

In addition to addressing crew health and safety, this research will also further our understanding of clinical gait syndromes. NASA and the National Institute of Aging (NIA) have recently entered into a collaborative agreement to pursue research topics of common interest. Both the 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.

FY96 Publications, Presentations, and Other Accomplishments:

Bloomberg, J.J., Reschke, M.F., Huebner, W.P., Peters, B.T., and Smith, S.L. Locomotor head-trunk coordination strategies following space flight. J. Vestib. Res., (in press).

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

LaFortune, M.A., McDonald, P.V., Layne, C.S., and Bloomberg, J.J. Space flight modifications of the human body shock wave transmission properties. Annual Meeting of the Canadian Society for Biomechanics, Vancouver, B.C., Canada, August, 1996.

McDonald, P.V., Basdogan, C., Bloomberg, J.J., and Layne, C.S. Lower limb kinematics during treadmill walking after space flight: Implications for gaze stabilization. Exp. Brain Res., 112, 325-334 (1996).

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

McDonald, P.V., Lafortune, M.A., Layne, C.S., and Bloomberg, J.J. Challenges to head stability after space flight. Society for Neuroscience Annual Meeting, Washington, D.C., November, 1996.

McDonald, P.V., Layne, C.S., and Bloomberg, J.J. Transmission of locomotor heelstrike accelerations after space flight: Implications for head movement control. American Institute of Aeronautics and Astronautics, 1996 Life Sciences and Space Medicine Conference, Houston, TX, March 5-7, 1996.

Smith, S.L., Peters, B.T., Layne, C.S., McDonald, P.V., and Bloomberg, J.J. The effects of visual target distance on head movement control during locomotion. 20th Annual Meeting of the American Society of Biomechanics, Atlanta, GA, October 16-20, 1996.

Smith, S.L., Peters, B.T., Layne, C.S., Mulavara, A.P., Pruett, C.J., McDonald, P.V., and Bloomberg, J.J. An integrated approach to the measurement of locomotion strategies in astronauts following space flight. Annual Houston Conference on Biomedical Engineering Research, Houston, TX, February, 1996.

Autonomic Mechanisms During Prolonged Weightlessness

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Funding:

Project Identification: E709
Initial Funding Date: 10/95
FY 1996 Funding: $249,700

Solicitation: 94-OLMSA-01
Expiration: 9/99
Students Funded Under Research: 3

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

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, we successfully integrated the two data acquisition systems required to perform this experiment. Our basic design includes two laptop computers, one of which has been outfitted with a sophisticated insert to measure hand-grip pressure, Valsalva pressure, respiratory flow, and end-tidal CO_2. The other laptop controls a myriad of measurement modules including ECG, arterial pressure, cerebral blood flow, body impedance, and microcirculation. Synchronizing the data acquired with these two similar but separate devices was not a trivial task; nonetheless, this was accomplished, the equipment was thoroughly tested, and it was sent into space. We had the unique opportunity to collect baseline data on two Russian cosmonauts involved in the DARA Mir '97 program. We are currently compiling and analyzing these data. We completed a ground-based study determining the influence of differential respiratory inputs on cardiovascular dynamics. These data represent the normal response of the autonomic nervous system to respiratory interventions, and will provide important insights into potential modulation of cardiovascular dynamics after exposure to prolonged weightlessness. Our preliminary work with the DARA Mir '97 mission, coupled with our preliminary data collected on the ground, will provide us with valuable experience and information critical to the success of the American training and data collection activities scheduled to begin soon.

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

FY96 Publications, Presentations, and Other Accomplishments:

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Eckberg, D.L. "High and low pressure baroreflexes" in "A Primer on the Autonomic Nervous System." Edited by: Robertson, D., Low, P., and Polinsky, R. Academic Press, San Diego, CA. 1996.

Eckberg, D.L. "Autonomic Nervous System Adaptations to Space Flight" in "Physiological Basis of Occupational Health: Stressful Environments." Edited by: Shiraki, K. and Yousef, M.K. SPB Academic Publishing/Amsterdam, 1996.

Halliwill, J.R., Taylor, J.A., and Eckberg, D.L. Impaired sympathetic vascular regulation after acute dynamic exercise. J. Physiol., vol. 495, no. 1, 279-288 (1996).

Halliwill, J.R., Taylor, J.A., Hartwig, T.D., and Eckberg, D.L. Augmented baroreflex heart rate gain after moderate-intensity exercise. Am. J. Physiol., vol. 270, R420-426 (1996).

Morillo, C.A., Wood, M.A., Eckberg, D.L., and Ellenbogen, K.A. "Diagnostic utility of mechanical, pharmacological and orthostatic stimulation of the carotid sinus in patients with unexplained syncope." 45th Annual Scientific Session of the American College of Cardiology, Orlando, Florida, USA, March 24-27, 1996.

Smith, M.L., Beightol, L.A., Fritsch-Yelle, J.M., Ellenbogen, K.A., Porter, T.R., and Eckberg, D.L. Valsalva's maneuver revisited: A quantitative method yielding insights into human autonomic control. Am. J. Physiol., vol. 271, H124-129 (1996).

Taylor, J.A. and Eckberg, D.L. Fundamental relations between short-term R-R interval and arterial pressure oscillations in humans. Circulation, vol. 93, 1527-1532 (1996).

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 1996 Funding: $ 164,128
Students Funded Under Research: 1

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: ARC

Task Description:

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

During this phase of these studies, the Investigators met to discuss and finalize the details of the flight protocols, with the assistance of personnel from Ames Research Center. During this time, it was determined that both flight experiments would be carried out on the NASA-5 (Mir 24) mission.

Continuing USDA approval was obtained to import *Trigonoscelis gigas* into the U.S. One hundred-twenty beetles were transported from Moscow to UC Davis to serve as subjects in ground-control and hardware-development studies.

At the University of California, Davis, Dr. Hoban-Higgins continued experiments to establish the light characteristics necessary for entrainment and phase shifting of this species.

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

FY96 Publications, Presentations, and Other Accomplishments:

Hoban-Higgins, T.M. Circadian rhythms and spaceflight, focusing on the NASA/Mir Beetle experiment. Science and Technology Week, American River College, April 1996.

Sleep and Vestibular Adaptation

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Funding:

Project Identification: E639c

Initial Funding Date: 7/95

FY 1996 Funding: $150,166

Solicitation: 94-OLMSA-01

Expiration: 9/98

Students Funded Under Research: 4

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Task Description:

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

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

During 1996, the final design of our protocol for the Night Headband Monitor (NHM) experiment was completed. This included integrating our protocol into an overall sleep research project, involving not only our laboratory but those of Drs. T. Monk and H. Moldofsky. The integrated protocol was tested in Toronto using subjects from NASA and the Canadian Space Agency. The final protocol calls for monitoring the sleep of astronauts and cosmonauts for three 12-night blocks preflight, three blocks inflight, and three postflight. These recordings, encompassing 36 nights of inflight sleep recording per subject, will represent the most extensive sleep monitoring ever conducted of sleep in microgravity.

During this year, mission hardware was assembled and, where necessary, existing hardware was redesigned for space flight and constructed. A newly designed head movement sensor capable of functioning in microgravity was designed, built, and tested in simulated microgravity conditions on board the KC-135. Software for Mir's

on board MIPS computer was written and tested to allow astronauts and cosmonauts to download and review sleep data from the NHM.

Crew training was carried out for the first of our two NASA/Mir missions. Both the prime and backup crews of the NASA 4/Mir 23 flight were trained, and in August, the first 12-day block of preflight baseline data collection (BDC) was run, with a total of five astronauts and cosmonauts wearing the NHM at night and dictating dream reports each morning. The sleep data showed relatively normal sleep in the five, but individual differences were present, which we will track through subsequent preflight BDC blocks and then use to look at individual differences in microgravity and postflight recovery.

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, though limb and head movements are observed. It is not known whether either the major posture shifts or head and limb movements are gravity sensitive, but it would not be surprising to find that they are. Indirect evidence comes from astronaut reports of bizarre sleep postures in space and of persistent limb elevations on awakening from post-flight sleep. Thus, gravity and microgravity may exert differential effects upon sleep posture, and these may, in turn, affect the quality and quantity of sleep and even of dreaming.

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

As the vestibular system is initially perturbed by entry into microgravity, is the illusion of dreamed movement changed? Can this change be tracked as adaptation occurs? What new baselines are established under prolonged exposure? Finally, what is the sequences of changes when subjects reenter gravity? We see prolonged space

flight in the Mir Laboratory as an ideal setting to assess vestibular adaptation via its effects upon the experience of fictive movement in REM-sleep dreaming.

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

FY96 Publications, Presentations, and Other Accomplishments:

Hobson, J.A. "Sleep: Behavior and cellular mechanisms" in "Encyclopedia of Neuroscience, 2nd Edition." 1996.

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Crew Member and Crew-Ground Interactions During NASA/Mir

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Funding:

Project Identification: E628
Initial Funding Date: 9/95
FY 1996 Funding: $186,000

Solicitation: 94-OLMSA-01
Expiration: 9/98
Students Funded Under Research: 1

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: MIPS System

Task Description:

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

The objectives of this study are to measure and characterize changes over time in a number of important interpersonal factors, such as tension, cohesion, leadership role, and the relationship between space crews and monitoring personnel on Earth. These objectives will be assessed during the NASA/Mir missions by having both the crew members and personnel in ground control complete subscales from three standard mood and interpersonal group climate questionnaires: Profile of Mood States, Group Environment Scale, and Work Environment Scale. Along with a critical incident log and an experiences questionnaire, these measures will be completed on a weekly basis pre-mission, during the mission, and post-mission. By using an interrupted time-series analysis and a number of predicted correlations, a test of the hypotheses related to the objectives of our study will be made and discussed. There are no results to report for the FY96 period since funding for this study began during the last week of FY95 and the missions under study were not launched until late August, 1996.

This was the first full year of funding, and our early efforts were aimed at start-up activities such as establishing a lab, developing administrative reporting procedures, and procuring necessary hardware. We immediately prepared for data collection by translating the measures into Russian, developing data collection procedures, and formalizing our collaboration with the Russian co-investigators. In addition, both American and Russian crews (n=14) and mission control (n=21) subjects were trained and/or familiarized to the study procedures and measures.

Baseline data were collected for the NASA 3/Mir 22 members and their back-ups, and by the conclusion of FY96, flight data collection was underway. A total of eight Americans and 13 Russian subjects participated this year.

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 under stress. In addition, the ability of people from previously opposing political blocks to engage in complex activities, such as undertaking a space mission, serves as a model for international cooperation on Earth. Thus, this research project will teach us a great deal about ourselves and our ability to relate with one another despite cultural and political barriers.

FY96 Publications, Presentations, and Other Accomplishments:

Kanas, N. "The interpersonal environment of the crew during the HUBES/Mir space simulation." European Space Agency HUBES Symposium, Paris, France, November 27-28, 1995.

Kanas, N. "Groups in isolation: Revelance to group therapy." American Group Psychotherapy Association Annual Meeting, San Francisco, CA, February 13-17, 1996.

Kanas, N. "Social and cultural factors affecting crews on long-duration space missions." NASA/American Institute of Aeronautics and Astronautics, Houston ,TX, March 5-7, 1996.

Kanas, N. "Psychosocial pressures affecting people in space." American Psychiatric Association Annual Meeting, New York, NY, May 4-9, 1996.

Salinas, G., Kanas, N., Charles, J., Baker, E. "Life sciences operations in space: Issues and impacts." Life Sciences and Space Medicine Conference and Exhibition '96, NASA/American Institute of Aeronautics and Astronautics, Houston ,TX, March 5-7, 1996.

Magnetic Resonance Imaging After Exposure to Microgravity

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Funding:

Project Identification: E586
Initial Funding Date: 3/95
FY 1996 Funding: $239,000

Solicitation: 94-OLMSA-01
Expiration: 2/96
Students Funded Under Research:

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Task Description:

Our measurements on the crew of SL-J demonstrated significant muscle-specific atrophy after only eight days in weightlessness. Our published bedrest studies have documented the degree of expected atrophy after four months of disuse. We will repeat these muscle measurements on the long-duration missions of Shuttle/Mir to determine the degree of protection provided by the Mir exercise program. Our bedrest studies have shown that when normal subjects are put in bed rest, partially unloading the spinal column, significant intervertebral disc expansion occurs. This expansion reverts to normal shortly after reambulation following bedrest lasting days to a few weeks. Longer duration bedrest (17 weeks) however, results in some residual expansion that remains for some time following reambulation. We have shown that eight days of weightlessness (SL-J) does not result in residual expansion 24 hours after landing. We speculate that disc expansion during flight may be causally related to the back pain reported to occur during flight and that longer duration space flight will result in residual disc expansion that may pose some risk of disc damage during the landing and early post-flight period. This disc expansion with back muscle atrophy may be causally related to the back pain experienced after long-duration space flight. Several space experiments have documented altered hematopoietic activity which may be related to cellularity changes in the bone marrow. This research will measure the intervertebral disc cross-sectional area, muscle volumes and spinal bone marrow cellularity of the crew members before and after the Shuttle/Mir flights.

The baseline and postflight data collection was completed on the NASA-2/Mir 21 crew. Analysis of the data is approximately 75% complete.

The Russian MRI unit was upgraded to perform proton spectroscopy. Because of problems with Russian customs, the spectroscopy upgrade to the Russian MRI unit did not occur in time for the Mir 21 crew measurements. We do have good spectroscopy data on NASA-2; we are continuing to collect additional spectroscopy data beyond the nominal 30-day postflight data collection point when crew member time permits.

The preflight and postflight data on the NASA-3 crew is complete except for requested extra spectroscopy data. The crew has indicated the possibility of volunteering additional spectroscopy measurements. The Mir 22 Russian crew has recently returned and the R+5 day data point was successfully acquired, including data on the German astronaut who spent 20 days on the Mir (German flight, Mir96). The preflight spectroscopy measurements on the Mir 22 crew, obtained by our Russian colleagues, was not accomplished successfully. We will try to have one of our representatives present during future data acquisition sessions as often as possible to avoid loss of data in the future. The analysis of the NASA-3/Mir 22 data is just beginning.

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 1-G will cause bone and muscle loss. The loss of bone and muscle with aging occurs in both men and women, resulting in a significant public health problem. Although the exact cause of bone and muscle loss with aging is not understood, one important risk factor is disuse. Men and women become less active as they grow older, and that may play an important role in the elderly and in patients immobilized for medical reasons. In addition, muscle atrophy is an important component of many disease states as well as aging; therefore understanding the role of disuse versus other causes is important for elucidating the physiological mechanisms of muscle atrophy. The relationship of muscle atrophy to muscle performance is not well understood. The LMS flight will examine decrements in muscle performance with measurements of muscle-specific atrophy.

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

FY96 Publications, Presentations, and Other Accomplishments:

Bakulin, A.V., Oganov, V.S., Schneider, V.S., Voronin, L.I., Murashko, L.M., Novikov, V.E., Shakelford, L.C., and LeBlanc, A.D. Changes of human bone mineral density after long duration space flights. 17th Annual Meeting of the International Gravitational Physiology, Warsaw, Poland, April 14-19, 1996.

LeBlanc, A.D., Schneider, V., and Shackelford, L. Bone and muscle loss after long duration simulated microgravity. 17th Annual Meeting of the International Gravitational Physiology, Warsaw, Poland, April 14-19, 1996.

LeBlanc, A., Lin, C., Rowe, R., Belichenko, O., Sinitsyn, V., Shenkman, B., Oganov, V., Shackelford, L., and Feeback, D. Muscle loss after long duration spaceflight on Mir-18/STS-71. Annual Meeting of the American Institute of Aeronautics and Astronautics, Houston, TX, March 5-7, 1996.

LeBlanc, A., Schneider, V., Shackelford, L.L., West, S., Oganov, V., Bakulin, A., and Veronin, L. Bone mineral and lean tissue loss after long duration spaceflight. American Society for Bone and Mineral Research, Seattle, Washington, September 7-11, 1996.

Schneider, V., Oganov, V., LeBlanc, A., Rakmonov, A., Taggart, L., Bakulin, A., Huntoon, C., Grigoriev, A., and Veronin, L. Bone and body mass changes during space flight. Acta Astronaut, vol. 36, 463-466 (1995).

Zerath, E., Novikov, V., Grynpas, M., Bakulin, A., Holy, X., LeBlanc, A., and Oganov, A. Consequences of a 11.5 day spaceflight on bone mineralization in the Rhesus monkey. 10th International Workshop on Calcified Tissue, Jerusalem, Israel, March 10-15, 1996.

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Human Circadian Rhythms and Sleep in Space

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Funding:

Project Identification: E639
Solicitation: 94-OLMSA-01
Initial Funding Date: 10/95
Expiration: 9/98
FY 1996 Funding: $ 145,000
Students Funded Under Research:

Flight Information:

Flight Assignment: NASA 4/Mir 23, NASA 5/Mir 24

Responsible NASA Center: JSC

Task Description:

The aims of this study are to evaluate the sleep, mood and activation, body temperature, and performance of crew members involved in long-duration Mir space missions in microgravity. Because of time constrains for our experiment, we cannnot study every day of the mission. Instead, three week-long measurement blocks will be studied (early, middle, and late mission). This will, we hope, allow determination of the period length at which circadian rhythms in subjective activation and body temperature run, in order to detect "free-running" behaviors and to determine their consequences in terms of the patterning of daily levels of sleep quality and duration, mood, activation, and performance efficiency over the mission. Sleep will be evaluated by computerized sleep diaries (pre- and post-sleep); circadian rhythms by oral temperature, mood and subjective alertness (five times per day); and performance by verbal reasoning and serial search tests given one time per day. Sleep diaries will also be requested on the two days following each measurement block (and longer if possible).

FY96 consisted of the orientation and task training of the crew for NASA-4/Mir 23 and NASA-5/Mir 24 missions. Software was developed, refined, and translated ino Russian. A large amount of time and effort was spent ensuring the proper translation of the software. The experiment will be performed aboard Mir during FY97.

Life on Earth has developed to be in tune with cycles of daylight and darkness that stem from our planet's 24h rotation. Like most other animals, human beings have a biological clock inside the brain which acts as a timekeeper. For diurnal creatures like ourselves, the clock prepares the body an 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. Thus, for humans the figure is about 24.3 - 25.0h, depending on the individual. This means that the circadian system requires time cues or zietgebers (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 zietgebers on the human circadian system, and will help us in providing useful coping strategies to night workers and those suffering from jet-lag.

FY96 Publications, Presentations, and Other Accomplishments:

Monk, T.H., Buysse, D.J., Reynolds III, C.F., Kupfer, D.J., and Houck, P.R. Circadian temperature rhythms of older people. Exp. Gerontology, vol. 30, no. 5, 455-474 (1995).

Monk, T.H., Buysse, D.J., Reynolds III, C.F., Kupfer, D.J., and Houck, P.R. Subjective alertness rhythms in elderly people. Exp. Geronology, vol. 11, no. 3, 268-276 (1996).

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 1996 Funding: $126,051

Students Funded Under Research: 7

Flight Information:

Flight Assignment: NASA 4/Mir 23, NASA 5/Mir 24

Responsible NASA Center: JSC

Flight Hardware Required: SSAS, GSC

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 the purposes of this work. Standard operating procedures, quality control samples, and confirmatory experiments will be employed to ensure reliable, high-quality data. Analysis will be performed using both a modified form of EPA-approved gas chromatography/mass spectrometry (GC/MS) methods and new techniques based on direct sampling ion trap mass spectrometry (DSITMS). Significant effort will be put into developing, testing, and demonstrating DSITMS techniques with the requisite sensitivity, selectivity, and speed for real-time monitoring of trace-level contaminants in air. The results of this research will provide detailed information on the types and concentrations of VOCs in the Mir environment. Moreover, the demonstration of new technology and comparison against proven methods will yield valuable information on the feasibility of its use for monitoring air quality in advanced life support systems.

The two major goals of this research are the analysis of VOCs in air samples from Mir Station using a modified form of EPA-approved GC/MS methods, and the development and testing of novel methods based on DSITMS. Significant progress was made towards both of these goals in FY96.

A number of tasks addressed the implementation and application of GC/MS methods to the analysis of Mir samples. These methods involve the use of a modified form of EPA-approved techniques to identify and quantitate VOCs in air. They involve the use of an air concentrator to isolate the VOCs from the bulk of air sample (i.e., nitrogen and oxygen), GC to separate the various components from one another, and MS to detect them.

In April 1996, The JSC Toxicology Lab forwarded four air samples collected during the Mir 19 mission to Pete Palmer. Although these samples were not originally intended for analysis as per the original goals of the research proposal, their analysis enabled the testing and validation of the instrumentation and methods and provided valuable experience in interpreting chemical composition data on the Mir environment. These samples were analyzed by Carla Remigi, a graduate student in the Department of Chemistry and Biochemistry at SFSU, and the resulting data were interpreted by Nuvia Alvarez, a high school student participating in this research through the NASA SHARP program, with Palmer providing training, supervision, and oversight. This research was documented in a report titled "Preliminary Results from the Analysis of Air Samples from the Mir 19 mission." The results compared well with those from the JSC Toxicology Lab on the same samples. Specific VOCs identified included chlorofluorocarbons (CFCs), aromatic hydrocarbons (i.e., benzene, toluene, xylenes), and siloxanes. Concentrations of the VOCs were well below their space maximum allowable concentrations (SMACs).

In June 1996, the JSC Toxicology Lab forwarded four air samples from the Mir 21 mission to Palmer for analysis. Analyses of these samples were successful and were documented in the "NASA-2/Mir 21 30-Day Operational Accomplishments Postflight Report." Data interpretation was still in progress at the end of FY96 and results will be documented in the 180-Day Preliminary Science Report and 1-Year Final Report for this mission.

In the spirit of total quality, the instrumentation, analytical procedures, and data analysis procedures for GC/MS analysis of Mir air samples will continue to be refined and improved. Towards this end, Warren Belisle installed and tested state-of-the-art instrumentation for analysis of air samples. This included a cannister cleaning and dilution system, an Entech air concentrator, and a Varian GC/MS instrument. Belisle's instrumentation is now operational and will be employed for analysis of air samples from all subsequent Mir missions. An ultrahigh resolution GC column has been obtained for efficient separation of the various VOCs in the Mir air samples. The use of an ion trap mass spectrometer has been shown to offer improved sensitivity compared to other mass analyzers, with results from this work indicating detection limits on the order of 1 part-per-trillion by volume for 50 mL air. Standards for a number of unexpected VOCs identified in the Mir samples have been ordered to improve the accuracy of quantitation for these compounds.

A number of promising results was obtained with respect to the research and development of new DSITMS methods for real-time air analysis. The goal of this work is to develop new technology for real-time monitoring of VOCs in air samples, complementing and/or obviating the use of conventional methods as described above which are both equipment intensive (i.e., requires an air concentrator and a GC) and time consuming (i.e., usually an hour is needed to analyze a sample and significantly more time is needed for data interpretation). This is accomplished by using an appropriate sample introduction system to bring the air sample into the ion trap followed by MS/MS analysis for direct identification of specific VOCs in an air sample. The results of this research show promise for eventual application to *in situ*, real-time analysis of VOCs on future space missions, as opposed to the off-line, ipso facto GC/MS analyses that are currently used.

Research into real-time monitoring of monoterpenes in air was performed by Keith Coffee and Liana Lee, two undergraduate students in the Department of Chemistry and Biochemistry at SFSU. These compounds are emitted by plants, are routinely monitored in space environments, and indeed have been identified in air samples from several Mir missions. A novel chemical ionization (CI) technique using acetonitrile as a reagent gas was shown to provide a strong molecular ion signal for these compounds. This represents a substantial improvement over conventional CI reagent gas systems, such as methane and isobutane, which result in more extensive fragmentation and hence a weak molecular ion signal for these same compounds. This new CI technique effectively concentrates the signal of the monoterpenes into one major ion that can then be subjected to MS/MS analysis. Although MS/MS can be used to definitely identify monoterpenes in air, these same data show that the individual monoterpene isomers cannot be differentiated from one another by their fragmentation patterns. These results point out a potential limitation of the MS/MS technique for unambiguous identification of isomeric VOCs in air and underscores the importance of developing selective MS/MS methods to positively identify the target compounds of interest. In defense of the utility of these MS/MS methods for life support

monitoring applications, it should be stated that differentiation and quantitation of closely related structural isomers may not be necessary given that these compounds behave similarly from a toxicology standpoint. Regardless, further research into other reagent gas systems and ion-molecule reactions to differentiate monoterpenes and other isomeric VOCs of interest will continue.

Two different sample introduction systems were evaluated by Palmer, Remigi, and Coffee with respect to monitoring CFCs and monoterpenes in air. The first is a continuous monitoring device that combines the air sample with helium and passes the resulting mixture through an open-split interface and then into the ion trap. The second is a discrete monitoring device using a valve with a fixed sample loop and does not require the use of helium as a buffer gas as normally required for operation of the ion trap. Both systems provided detection limits on the order of 50 parts-per-billion by volume (ppbv) in MS, selected ion monitoring (SIM), and MS/MS modes of operation, with analysis times on the order of seconds for a specific VOC. Results of this research were presented at the 1996 Conference of the American Society for Mass Spectrometry and are being formalized into a manuscript for publication in a peer-reviewed journal. They have also led to the identification of a number of improvements to these sample introduction systems that will be explored in FY97.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Palmer, P.T. Recent advances in ion trap mass spectrometry and application to *in situ* monitoring of the Earth's atmosphere." Department of Chemistry and Biochemistry Seminar, California State University at Fullerton, California, March 28, 1996.

Palmer, P.T. Recent advances in ion trap mass spectrometry and application to *in situ* monitoring of the Earth's atmosphere. Lawrence Livermore National Laboratory, Livermore, California, March 27, 1996.

Palmer, P.T. Real-time air monitoring via direct sampling ion trap mass spectrometry." Society of Western Analytical Professors, San Francisco State University, San Francisco, California, February 2, 1996.

Palmer, P.T., Wong, C.M., Yost, R.A., Yates, N.A., Griffin, T.M. "Advanced automation of ion trap spectrometry - New opportunities for real-time, autonomous analysis" in "Artificial Intelligence Applications in Chemistry." Edited by: Brown, S. Wiley, pp 25-60, 1996.

Remigi, C., Palmer, P.T., Karr, D. Real-time monitoring of chlorofluorocarbons in air via direct sampling ion trap mass spectromery." Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, Oregon, May 12-17, 1996, p. 523.

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 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir 1B

Responsible NASA Center: JSC

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 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; and (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.

Data Collection

•Mir-21 pre- and postflight data were collected on two subjects. Three preflight (60, 45, and 30 days before launch), five postflight (3, 8, 15, 64, and 128 days after landing) for data collection #1, and three postflight (1, 65, 129) for data collection #2 were performed.

•Pre- and postflight data were collected on the NASA-2 subject. Two preflight (60 and 30 days before launch) and eight postflight (0, 1, 4, 5, 11, 19, 35, and 64) were performed.

•All Mir-21/NASA-2 data were lost inflight. The Gaze experiment was never performed because of a MIPS-3C failure.

Data Analysis Summary

Data analysis continues.

Preliminary Science Findings

Most, if not all, neurosensory and sensory motor functions that support our orientation in three-dimensional space are disrupted or modified by sustained exposure to microgravity. These disruptions have been manifested by changes in the postural control mechanisms, locomotion, the ability to maintain the head (or sensory platform holding the eyes and vestibular system) stable, the degradation of visual and visual/vestibular pursuit, the ability to acquire select visual targets, the ability to smoothly pursue a moving target, the vestibular assisting optokinetic system, and sensory coordination.

The present gaze experiments have suggested that the gaze holding mechanism responsible for maintaining the eye on target may be affected. Specifically, evidence from Mir-21 suggests that the mechanisms within the brain responsible for maintaining gaze on a selected target are no longer functional, and that the modifications of the gaze holding mechanisms are due to prolonged exposure to microgravity. Importantly, these changes may be adaptive in nature.

To understand the changes taking place, it is helpful to consider the way that the brain encodes eye movement signals when there is a desire to move gaze from one visual location to a new target. Once an individual has determined that she/he will shift gaze from one place in the visual field to another, neurons in the brainstem encode the eye movement signal with a proportional shift in the frequency of neuronal spike discharges necessary to move the eye from one position in the orbit of the skull to a new position. These discharges vary linearly with eye position during fixation. In addition, during conjugate movement of the eyes, the brainstem motoneurons modulate their discharge in proportion to eye velocity. Therefore a mixture of position and velocity neuronal discharges is necessary to move the eye from an existing point of interest to a new target.

A velocity command is required for a saccade (the fast movement of the eye) to move the eye to a new target. The short lead burst neurons, located primarily in the pons, provide the needed velocity command signal by discharging at a high frequency. The output of these burst neurons is preceded by a brief lack of neuronal activity in omnidirectional pause neurons relieving the inhibition that these cells exhibit over burst cells. This chain of events results in a phasic contraction of the eye muscle via the ocular motoneurons that overcome mechanical properties of the eye, and reposition the eye in the skull's orbit. At the end of the saccade a new position command is generated sending a neural signal via a neural integrator causing a tonic contraction of the eye muscles to hold the eye in the new position against any mechanical restoring forces existing in the physical properties of the eye system that would attempt to move the eyes back to a neutral position.

If the neural integrator functions correctly, the eye will remain deviated in its new position. If, however, the neural integrator is faulty, it will "leak" similar to a capacitor losing its charge. As the integrator leaks, restoring forces will cause the eye to drift back in the orbit towards its neutral position. The rate at which it returns can be described by a time constant (i.e., the time it takes for the eye to drift 63% of the way back to a predetermined null point). The leakier the integrator, the shorter the time constant.

The neural integrator is believed to be composed by structures in the brainstem (e.g., nucleus prepositus hypoglossi and the medial vestibular nucleus: at least for horizontal eye movements), and the state of the neural integrator is set by structures in the cerebellum. It is known that lesions of the flocculus and paraflocculus, for example, make the neural integrator deficient (leaky). The function of the cerebeller floccular sites is to improve the performance of a normally leaky neural integrator by using a positive feedback loop where the time constant of the neural integrator is improved (longer time constant). What is important in this interactive context is that the concept of sensory integration within the brain and mechanisms for environmental adaptation.

NASA's Mir Gaze experiment (E647), "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 ensure the safety, health, and well being of future astronauts.

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: 9/95
Expiration: 9/ 96
FY 1996 Funding: $95,000
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Task Description:

The changes in immune function which occur during space flight potentially expose the crews to an increased risk for development of illness. Decreased cellular immune function has been repeatedly documented after space flight and confirmed during flight by *in vivo* delayed-type hypersensitivity testing. The mechanisms of these responses and the involvement of the different arms of the immune system are currently unclear. Our hypothesis is that space flight will cause a decrease in humoral immune function similar to that observed with the cell-mediated immune system. To test this hypothesis, crew member volunteers will be immunized with polysaccharide antigens and the production of immunization specific antibodies will be determined. The immune responses generated during flight will be compared to responses from a synchronous ground-based control group. Assessment of *in vitro* B cell function will also be performed. A thorough understanding of the immune system function during space flight is critical to the assessment of crew health risks.

An initial measurement of the pre-immunization antibody titers for the preflight and inflight samples has been performed for the 4 most common pneumococcal isotopes. Analysis of the remaining samples will be performed following STS-84 landing and collection of the NASA-4 samples. The samples will be analyzed in batch with NASA-2, NASA-3, NASA-4 and their age/sex matched ground controls. The samples will be assayed for antigen-specific antibody levels against the 23-valent pneumococcal vaccine. Data reduction and statistical analysis has not been initiated since sample collection for batch analysis is not yet complete.

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
Initial Funding Date: 7/96
FY 1996 Funding: $237,178

Solicitation: 94 OLMSA-01
Expiration: 7/97
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B
Responsible NASA Center: JSC

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.

Thirteen reclaimed water, twelve ground-supplied water, and thirteen humidity condensate samples have been collected from Mir 18 - Mir 21. Findings from the analysis of the reclaimed water samples show that the water met all requirements of the Joint NASA/RSA spacecraft water quality standards. It is noted, however, that the NASA requirements for TOC, turbidity, and occasionally for total phenols were exceeded. In addition, the regenerated water did not meet U.S. EPA water quality standards for methylene chloride in one instance. Although the exceedance of these specifications is noteworthy, the reclaimed water is considered potable. The ground-supplied water is also considered potable, although it exceeded the NASA requirements for TOC, turbidity, and ammonia and the U.S. EPA maximum contaminant levels for chloroform and methylene chloride. As noted, selected parameters were exceeded, but not to a degree which would adversely affect the potability of the water.

The humidity condensate had significantly lower levels of alcohols and organic acids as compared to the shuttle condensate. TOC levels were also much lower than levels previously measured in the shuttle. Compounds detected in the Mir condensate which were much higher than those measured on the shuttle include acetaldehyde, acetone, 1,2-ethanediol, and 1,2-methoxy-2-propanol. One source of 1,2-methoxy-2-propanol is from the Sharpie® markers used onboard Mir. Also, the high levels of 1,2-ethanediol were due to a leak of this compound from a coolant loop in the Mir core module. The ability to collect humidity condensate and subsequently analyze the samples on the ground were critical in planning the operational response to this leak.

This research will provide benefits in the areas of methods development for the analysis of drinking water, advanced technologies for the treatment of wastewaters, and increased knowledge of potable water contaminants. Improvements in methods development as a result of this experiment will potentially increase the sensitivity of organic analyses 10 fold over present techniques. These improvements will allow more complete characterization of potable water, accounting for most organic constituents, even those at extremely low levels. In addition, by adapting techniques for treating spacecraft waters, the development of better wastewater treatment technologies on Earth will be supported.

Bone Mineral Loss and Recovery after Shuttle/MIR Flights

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Funding:

Project Identification:
Initial Funding Date: 4/95
FY 1996 Funding: $150,000

Solicitation: 94-OLMSA-01
Expiration: 9/00
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

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 1-G. Knowledge of the rate and degree of bone recovery is important not only for NASA, but for clinical investigators interested in reversing the effects of osteoporosis. This proposal will measure the space flight-induced bone loss of the twelve crew members of the Shuttle/Mir flights and follow the recovery with bone mineral measurements every six to twelve months for up to three years or until full recovery has occurred. In order to gain information on the role of muscular fitness with respect to bone loss and recovery, muscle strength testing will be performed at the same time points as bone mineral measurements. Muscular fitness will be used as an indicator of a crew member's level of load-bearing physical activity throughout the study. Serum and urinary markers of bone metabolism will be measured pre- and post-flight in order to provide information regarding the altered metabolism of bone resulting from long-duration flight. This information will complement the bone density results and may shed light on the mechanisms involved in disuse bone loss and subsequent recovery.

All scheduled data collections were successfully completed. These data collections included the final preflight measurements of the NASA-2/Mir-21 crew; R+0, R+5, and R+14-day measurements of the NASA-2/Mir-21 crew; preflight and R+0, R+5, and R+14-day measurements of the NASA-3/Mir-22 crew; and preflight measurements of the NASA-4/Mir-23 crew. Data collections consisted of bone densitometry (DEXA) scanning,

muscle strength testing by LIDO isokinetic dynamometry, and blood and urine collections for the measurement of serum and urinary markers of bone metabolism. A preliminary analysis of pre- vs. postflight bone density and muscle strength has been performed on four NASA/Mir crew members (including one crew member of the Phase IA flight). These preliminary results appear similar to results obtained previously on 18 long-duration cosmonauts, both in terms of the variability in bone loss among individuals, as well as the site-specific variability in bone loss within a given individual. Changes in lean tissue mass in the NASA/Mir crew members also appeared to be similar to those changes documented in the 18 cosmonauts. Analysis of strength data and bone marker data on the NASA-2/Mir-21 crew is nearing completion at this time. Progress on this study within the past year has not indicated a need to change any of the functional objectives or data collection procedures in the future.

The study is now entering perhaps its most important phase – that of documenting the degree and rate of bone recovery in the post-flight period. In support of this prime objective, R+6 month bone density measurements were recently completed on one of the NASA-2/Mir-21 crew members, and we hope to obtain similar measurements on the remaining crew members from this flight, as well as crew members on the subsequent Phase IB flights. R+6 month measurements will be followed up by yearly measurements (up to three years postflight) or until complete recovery is confirmed. Muscle strength measurements in the post-flight period will be used as an indicator of muscular loading activity and may shed light on the bone recovery process.

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:
Initial Funding Date: 10/94
FY 1996 Funding: $200,000

Solicitation: 94 OLMSA-01
Expiration: 9/98
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA-Mir-1B, Mir-22/NASA-3
Responsible NASA Center: JSC

Task Description:

Muscles that are not used lose their strength. In addition to the loss in muscle mass during and after space flight, there is a loss of muscular fitness. This response is similar to observations with prolonged immobilization, such as being bedridden. When reduced fitness occurs, decreases in strength, endurance, tone, and efficiency result. Investigators for this experiment hypothesize that being in a weightless environment results in non-uniform changes (e.g., extensors > flexors, legs > arms) during flight with a slow readaptation to preflight levels upon return to Earth.

One objective of this experiment is the evaluation of how skeletal muscle performance and characteristics adapt during long duration space flight. Investigators then compare post-flight response with preflight values to determine how long it takes (and what mechanisms are used) to readapt to Earth's gravity. The tests protocols included: (1) muscle strength, endurance and tone, (2) neuromuscular efficiency, (3) voluntary and evoked contractions, and (4) integrated muscle performance testing on a passive treadmill. These protocols were performed before and after Mir-18 and helped evaluate the efficacy of the Russian Countermeasures. Evaluating the metabolic cost of passive running on the treadmill during STS-71 helped determine the extent of the postflight change in performance.

The following tasks were conducted during fiscal year 1996:

- Completed experiment documentation manual.
- 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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No Co-Is Assigned to this Task

Funding:

Project Identification: E613

Solicitation: 94-OLMSA-01

Initial Funding Date: 1/96

Expiration: 12/96

FY 1996 Funding: $193,955

Students Funded Under Research:

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

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 space flight, 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.

By the end of FY96, one American had completed her time in space, and the two Russians were within a month of completing their stay in space. The other American had completed nearly four months in space. The preflight data collection sessions met the planned schedules. Inflight, between one and three data points were collected middle and late in the mission. Analyses are still in progress.

The question of whether humans can truly adapt is of both practical importance and of general biological interest. If a 'mild' but chronic stress response continues with its associated energy and protein wasting, long-term space missions to destinations such as Mars become very problematic unless effective countermeasures are developed. If the stress response is short and finite, indicating true adjustment to the new environment, then the problem is known, is limited in duration, and is not serious. Space flight confronts humans with a totally novel situation. Is there enough flexibility in the genetically determined response to stress that humans can adjust to stresses for which there can be no pre-programmed specific response?

Microbial Interaction in the Mir Space Station Environment

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Funding:

Project Identification: E703

Initial Funding Date: 01/96

FY 1996 Funding: $126,789

Joint Agency Participation: None

Solicitation: 94-OLMSA-01

Expiration: 10/98

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NASA-MIR-1B

Responsible NASA Center: JSC

Task Description:

The long-term goal of this project is to understand the nature of microbial behavior in closed, long-duration space flight systems. This will be accomplished in part by applying techniques of molecular genetics. These studies in conjunction with other approaches should provide the information and methodology required to (1) set standards for microbial exposure, (2) develop in flight assays for microorganisms that determine if conditions are within prescribed standards, and (3) implement appropriate procedures to control microbial concentrations when needed.

The hypothesis is that microorganisms have been introduced into the Mir space station over the eight years it has been in orbit from the natural microbial flora of crew members as well as payloads and other on-board sources. These microbes have been dispersed throughout the space station and have settled into specific environmental niches. A unique natural selection for microbes that can flourish in the Mir environment has occurred during this time. Accurate assessment of this unique microbial ecology is critical for understanding the behavior of microorganisms that will inevitably be introduced into any closed-space environment. To this end, we will perform a study using restriction endonuclease digestion and pulsed field electrophoresis to fingerprint microorganisms from space flight missions. These samples will also be used to develop a faster assay based on the polymerase chain reaction. These techniques will allow microbes introduced by the crew to be distinguished from those already present on Mir. It will also be possible to tell if crew members pick up microbes from the space station environment and whether crew members transfer microbes themselves. Microorganisms that are identified by this procedure as being endogenous to the space station will then be assayed for various properties such as antibiotic resistance, adhesion to surfaces, DNA repair capacity, stress responses, growth in poor media, and secreted proteins and compared to standard strains. This will determine if any special properties are selected for among microbes that persist in the space station.

The initial technology development phase of this project has largely been completed. We used 112 strains of *Staphylococcus aureus* for this purpose. Five strains were previously obtained from space shuttle missions, 90 strains were from Mir 18 and Mir 19, 15 strains were clinical isolates from local hospitals, and two strains were

well-characterized laboratory strains. These were used to compare our previously described pulsed field gel electrophoresis analysis of whole genome fingerprints to a new method developed using repeated sequence PCR for fingerprinting. This latter method is faster and more convenient. Initial studies indicated that, while not as sensitive as the former method, the REP-PCR approach had sufficient sensitivity to allow accurate strain comparison on large scale. This was demonstrated for the Mir 18/19 samples.

The results of this analysis showed that, while microbial transfer among crew of short-duration Space Shuttle missions is rare, there is considerable transfer among the crew of long duration Mir missions, starting during the training period. It was also observed that some crew members are carriers of *Staphylococcus aureus* (but show no symptoms of infection), other crew members are susceptible to colonization during training, and some crew members are resistant to colonization by *S. aureus*.

These extremely positive results from this new technology encourage us to investigate application to tracking of other microorganisms.

Tracking of microorganisms is an activity required in numerous critical situations on Earth. These include epidemiological investigations of local outbreaks, for instance in day-care centers, hospitals, and other closed environments such as office buildings. Such microbial tracking is not carried out often enough due to the requirements for specialized equipment and technical know-how that is not always present. The development and successful application of the REP-PCR methodology should simplify and expedite the tracking of microbes by DNA fingerprinting and make this type of investigation much more generally applicable. The Mir space station missions provide an excellent opportunity to develop and test methodology to follow microbial transfer in a situation that is of great interest to the space community, where the need to control infectious diseases is critical in a closed environment where there will not be access to extensive medical care. At the same time, this methodology, once proven, can be readily extended into the situations on Earth alluded to above.

Renal Stone Risk During Long Duration Space Flight

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Funding:

Project Identification: E651

Solicitation: 94-OLMSA-01

Initial Funding Date: 1995

Expiration: 1998

FY 1996 Funding: $80,000

Students Funded Under Research: 0

Joint Agency Participation: N/A

Flight Information:

Flight Assignment: NASA-Mir-1B

Responsible NASA Center: JSC

Flight Hardware Required: Urine Collection Device (UCD), Bar Code Reader (BCR)

Task Description:

This investigation was manifested for the Mir-21, NASA-2, Mir-22 and NASA-3 missions. Preflight, in-flight and postflight urine and data analysis are completed for the Mir-21/NASA-2 crew members. Samples and dietary data have been received for the Mir-22/NASA-3 crew members and data analyses is continuing. Upon completion of the sample analyses, the potential for renal stone formation will be evaluated. Data from these missions will be combined with the results from the Mir-18 mission and potential countermeasures will be assessed to decrease the risk for renal stone formation.

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 crewmembers exposed to microgravity. The first phase of this investigation will assess the direct effects of microgravity on this potential during long duration space flight. Following this assessment, proven Earth-based therapies will be recommended to protect the health and well-being of the crewmembers.

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. This advanced technology is scheduled as a technology demonstration on the NASA-6/NASA-7 and Mir-25 missions.

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 1996 Funding: $
Students Funded Under Research: 3
Joint Agency Participation: NIH/National Institute of Neurological Disorders and Stroke

Flight Information:

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

Responsible NASA Center: ARC

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 FY96, the primary tasks for this proposal were to: 1) further refine the experimental conditions for implanting the osmotic pumps in nursing Dams in order to make suckling neonatal rats thyroid deficient; 2) use this technology and demonstrate its effectiveness in the Experimental Verification Experiment; 3) perform a ground-based experiment to define the developmental pattern of MHC isoform expression in rodent skeletal

muscle of normal and thyroid deficient rodents; and 4) continue the development of techniques to quantitatively asses MHC mRNA expression in small samples of skeletal muscle using the Reverse Transcriptase Polymerase Chain Reaction (RTPCR) Technique.

Osmotic Pump Implantation Accomplishments and Verification Testing Results:

We have succeeded in implanting osmotic pumps into the peritoneal cavity of Nursing Dams with sufficient quantities of propylthiouracil (PTU) being released to make the suckling pups hypothyroid, based on several lines of criteria indicative of a hypothyroid state (blood thyroid analyses, body mass, muscle and heart mass, and cardiac myosin profiles). This procedure was successfully used in the EVT in November 1996 at Ames Research Center and provided data to support our technology (as submitted in the preliminary EVT report). However, two of the 9 implanted animals, broke their skin stitches and caused concern by NASA project scientists. Presently, we have successfully tested new suturing techniques involving a double matrise technique in combination with wound clips to fully ensure that the superficial skin sutures are fully competent. Thus, we feel that this component of the project is completed and in place to meet our mission objectives.

Ground-Based Study on MHC Development in Neonatal Rats:

We have performed a time-course analysis of MHC expression in fast and slow rodent skeletal muscles at critical stages of development in both normal and thyroid-deficient animals. Our findings suggest that in 5-7 day-old rats, MHC expression is predominated by expression of the immature embryonic and neonatal MHC isoforms in both slow-soleus and fast-plantaris muscles, with the latter demonstrating that it is only 15% matured relative to the adult MHC phenotype while the soleus is 50% matured to the adult state. During the subsequent 3-4 weeks of development, the embryonic/neonatal MhC are repressed completely in both the fast and slow muscles and replaced by the adult fast isofoms and the adult slow isoforms in the plantaris and soleus muscles, respectively. Deprivation of thyroid hormone markedly blunts and slows that maturation process in the fast muscles while causing an over abundant expression in the slow solues muscle. Thus, our findings are consistent with the original hypothesis that an intact thyroid state is necessary for the establishment of the normal adult phenotype, as well as maintaining the normal growth, i.e., mass of the skeletal muscle system. We are in the process of writing a full manuscript on these findings which will be presented at the 1997 Experimental Biology.

Progress on RTPCR Analytical Techniques:

We have been successful is establishing a quantitative technique using RTPCR so that we can simultaneously analyze the six primary MHC mRNAs in developing and adult skeletal muscles, including the embryonic, neonatal, adult slow (I), and adult fast type IIa, IIx, and IIb forms. We have designed the assays to include an internal standard segment of DNA that contains the necessary primers for the MHC reaction so that we have an internal reference so that the reaction products can be normalized for reaction efficiency thus enabling a given set of samples to be compared to one another in terms of the relative expression of the various MHC mRNAs. Our approach is as reliable and as accurate as the method of Northern blot analysis that is the primary standard assay currently used. However, our RTPCR assay can be done in one fifth the time and using less costly materials. We have submitted a paper describing our approach and its applicability to analyzing the type of samples we will be generating on the Neurolab Mission to the Journal of Applied Physiology.

In this flight project, we will be addressing fundamental issues concerning the role of gravity and in particular the interaction of gravity forces and thyroid hormone in the regulation of the pattern of skeletal MHC expression in rodent antigravity and locomotor muscle. Previous work on both ground-control and space-flight animals suggests that gravity plays a pivotal role in dictating the muscle's contractile protein phenotype. We feel that this dependency on gravity to control the properties of muscle will be even more dramatic when examined in the context of muscle development. The adult phenotype for contractile and hence functional capability evolves during post-natal development. We believe that gravity may be essential for establishing the expression of slow MHC in muscle fibers, which is essential for antigravity function. That is, in the absence of gravity during neonatal development, the slow MHC gene will not be turned on sufficiently to establish this property. Also,

since thyroid hormone appears to be essential for the normal development of muscle mass and contractile phenotype, we want to manipulate thyroid state as well in ascertaining the interaction of thyroid hormone (or its absence) and that of gravity on the muscle maturation process.

These experiments will for the first time delineate how gravity impacts an important developmental and maturation process which affects muscle mass and locomotor performance. While this work will not address a specific disease per se, we feel that the environment of weightlessness creates a disease-like process such as muscle wasting (atrophy). The research in this proposal will address this topic indirectly by examining the potential retardation of muscle growth, differentiation, and gene expression in young animals.

FY96 Publications, Presentations, and Other Accomplishments:

Baldwin, K.M. Isomyosin expression in developing skeletal muscle: Effect of thyroid state and innervation. South West Chapter of American College of Sports Medicine Meeting. Las Vegas, November, 1996.

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 1996 Funding: $321,341
Students Funded Under Research:
Joint Agency Participation: NIH/National Institute of Neurological Disorders and Stroke

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: LBNP, handgrip valsalva and cold press. dev., ECG transcranial doppler, etc.

Task Description:

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

The experimental design represents an integrated approach to the testing of this primary hypothesis. The following questions will be answered: 1) Does efferent sympathetic nerve activity increase appropriately in response to baroreflex and non-baroreflex-mediated stimuli during and after space flight? 2) Can integrated clinical tests of autonomic function detect functional impairment, and can they be used to characterize the time course of adaptation to microgravity? 3) Does regulation of the cerebral circulation change in parallel with or independent of the regulation of the systemic circulation? 4) Can advanced mathematical models of neural control including both linear and non-linear dynamics be developed to gain insight into the integration among neurocirculatory variables and control mechanisms? A series of well-defined physiological stimuli has been defined, including lower body negative pressure, a cold pressor test, isometric exercise, Valsalva, and controlled breathing. Responses are characterized by multiple measurements including heart rate, continuous finger arterial pressure and direct recording of muscle sympathetic nerve traffic.

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, validated, and approved by the human use committee at JSC. Instrumentation has been defined, tested, and integrated. Detailed plans for supporting ground-based studies and for crew training have been defined and implemented. Crew training has included microneurography.

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.

Space Flight, Stress, and Neuronal Plasticity

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Funding:

Project Identification:

Solicitation: AO 93-OLMSA-01

Initial Funding Date: 8/96

Expiration: 8/97

FY 1996 Funding: $80,311

Students Funded Under Research: 2

Joint Agency Participation: NIH/National Institute on Aging

Flight Information:

Flight Assignment: Small Payload (TBD)

Responsible NASA Center: ARC

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.

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 established the ground-based studies and refined protocols for eventual application to space-flight animals. The necessary reagents and equipment have with one exception been obtained or developed. Baseline studies are now being done on control animals and steriod treated animals. An effective procedure for the administration of corticosteroids and drugs has been developed and will be refined further in the coming months. We have established the validity of ELISAs for quantitiative analyses of

cytoskeletal proteins in different brain regions. Signficant differences have been found in the levels of several cytoskeletal proteins in steroid treated animals. These studies are being extended to additional markers and regions of the brain. The Golgi impregnation method for visualizing neuronal and glial morphologies is being optimized, and initial data collection on cell shape has begun. Immunocytochemical studies and quantitative *in situ* localization of mRNA in brain sections are being initiated. Previous work on presynaptic function in the squid giant synapse is still underway.

The studies supported by this grant are intended to look at the effects of physiological stress on neuronal function and neuronal architecture. Previous studies have shown a number of deleterious effects on neuronal functional architecture associated with chronic stress. The conditions of space flight can result in stress of unusual duration, but physiological stress is commonly associated with a wide range of human activities. Many stress-related medical conditions have been documented. Since many of these changes appear similar to changes associated with the aging nervous system, these studies may also illuminate the mechanisms that lead to decrements in neuronal function with aging. The goals of these studies are: 1) to understand the molecular basis of neurological changes associated with stress, and 2) to devise treatments that can minimize deleterious changes in neurological function associated with chronic physiological stress.

FY96 Publications, Presentations, and Other Accomplishments:

Brady, S.T. A kinesin medley: Biochemical and functional heterogeneity. Trends Cell Bio., 5, 159-164 (1995).

Brady, S.T. Motor neuron diseases: Interfering with the runners. Nature, 375, 12-13 (1995).

Brady, S.T. and Sperry, A.O. Biochemical and funtional diversity of microtubule motors in the nervous system. Curr. Opinion Neurobio., 5, 551-558 (1995).

Elluru, R., Bloom, G.S., and Brady, S.T. Fast axonal transport of kinesin in the rat visual system: Functionality of kinesin heavy chain isoforms. Mol. Bio. Cell, 6, 21-40 (1995).

Sickles, D.W., Brady, S.T., Testino, A., and Wrenn, R.W. Direct effect of the neurotoxicant acrylamide on kinesin-based microtubule motility. J. Neurosci. Res., 46, 7-17 (1996).

Sperry, A.O., Cyr, J.L., and Brady, S.T. The kinesin superfamily of molecular motors. Adv. Mol. Cell Bio., 12, 165-190 (1995).

Stenoien, D. and Brady, S.T. Inhibition of kinesin function by an antibody to a conserved epitope on kinesin light chains. Mol. Biol. Cell, (in press).

Microgravity Effects on Developing Vestibular Afferents

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 7/95

Expiration: 6/99

FY 1996 Funding: $84,787

Students Funded Under Research:

Joint Agency Participation: National Science Foundation

Flight Information:

Flight Assignment: ARF-2 and Small Payload (TBD)

Responsible NASA Center: ARC

Task Description:

This project will examine effects of the gravitational environment on the development of specific neuronal connections between vestibular sensory organs and their central nervous system targets in the zebrafish, *Brachydanio rerio*.

The proposed flight experiments, involving examination of primary vestibular afferents of zebrafish embryos raised in microgravity, will help determine the effects of altered patterns of neuronal activity on the development of connections in the vestibular system. In addition, these experiments may reveal an anatomical substrate for the observed plasticity in swimming behavior of fish seen during space flight.

The development of organotopic specificity in the primary vestibular afferent projection to the vestibular nuclei will be studied in zebrafish raised in three different environments: microgravity, 1-G centrifugation during flight, and 1-G ground-based control conditions. Lipophilic-dye fiber-tracing techniques will be used to label populations or single axons in specimens fixed at different ages. The pattern and extent of axonal growth of afferents from each vestibular sensory organ will be examined both in whole mount using confocal microscopy and in cryostat sections. These experiments will be the first to study the effects of microgravity on the development of the neuronal connections underlying vestibular senses, as well as to document the normal development of these connections. Flight experiments will provide data on the relative role of patterns of neuronal activity versus inherent positional cues and tropic factors in the development of specific connections in the vestibular system.

During the past year, this project has undergone a change in flight hardware at NASA's request. It was previously planned that the zebrafish embryos would be flown in Egg Chamber Units (ECUs); now we plan to fly the embryos in the Canadian Space Agency (CSA) Aquatic Research Facility (ARF).

This change in hardware has one major advantage as far as science is concerned — the ARF allows for a 1-G in-flight control as was originally requested in the Neurolab proposal for this project. This in-flight control will

make it possible to determine precisely the effects of microgravity on the developing vestibular afferents in the zebrafish, rather than merely being able to study the effects of space flight (including non-gravity components such as vibration, acoustic noise, radiation, changes in carbon dioxide, pH, etc.) on the system.

However, the new hardware has also necessitated some new compromises on the proposed science. Because the ARF can only hold six specimen chamber units (SCUs) (each containing two very small aquaria (SCAs)) at each gravitational condition (0-G and 1-G), and because CSA and NASA have agreed that three PIs will fly on each ARF mission, the number of fixation time points which will be possible during the flight has been reduced from the seven time points (approved at the end of the Neurolab Definition Phase and already reduced from the originally requested 14 time points) to at most four time points.

Perhaps even more critically, the small size of the SCA (35 mls volume, as compared to 150 mls in each ECU) combined with the decrease in the allowed number of chambers will severely limit the total numbers of zebrafish embryos which can be flown. An additional problem caused by the change in hardware is that the original proposal called for fixation of specimens in-flight using 4% paraformaldehyde. The only fixative approved by CSA for use in the ARF, however, is 0.5% glutaraldehyde. This is due to safety concerns arising from the fact that the automatic fixation process used by the ARF involves burning through a nylon cord to release the spring which pushes a blade through a septum, allowing fixative to enter the SCA. Glutaraldehyde was chosen as a safe fixative in this system because it has a relatively high flash-point, making it unlikely to ignite when the nylon cord is heated to its melting point.

During the past year, our work has been concentrated on two areas: First, we have purchased and set up the laboratory equipment necessary for performing the labeling and examination of the primary vestibular afferents in experimental and control zebrafish embryos. We now have the necessary facilities in place to manufacture pipettes used in the labeling procedure, to perform iontophoretic injections of fluorescent lipophilic dyes into the sensory epithelia of the developing inner ear, and to examine the labeled afferents resulting from these injections. We are still waiting to hear from NSF regarding funding of a grant we have submitted (along with several CO-Is at UCD's Center for Neurosciences) which would fund the confocal microscope which is necessary for the performance of our experiments and which was refused funding by NASA during the review of the initial Neurolab proposal. Second, we have begun extensive experiments designed to test the feasibility of using the ARF hardware to carry out the proposed science. These studies have focused on two areas: survivability of the embryos in the chambers, and possible fixation paradigms.

We were only able to obtain the SCUs from CSA in October 1996, so survivability studies are still being performed. These studies are designed to determine the largest number of embryos which can be loaded into a given SCA that will survive for the duration prior to their planned in-flight fixation time. Because we have only been able to obtain from CSA one SCU which has all three levels of containment (and which it is therefore possible to use to test the survival in the configuration which will be used during flight), and because we are planning on a flight of 9-14 days in duration, these tests will take some considerable time to complete.

In testing the compatibility of the CSA-approved fixation procedures with our experimental design, we have run into two possible problems. We did find that 0.5% glutaraldehyde does appear to produce good fixation of our specimens at the gross level. Unfortunately, glutaraldehyde produces fluorescence of the tissue in similar wavelengths to the fluorescence of the dyes we use to label the primary vestibular afferents in our studies. Therefore the glutaraldehyde fixation interferes with our ability to observe the labeled afferents which are the basis of all of our experiments. We are currently working with Chroma to try to produce fluorescent filters which will block the glutaraldehyde fluorescence while still allowing us to see our labeling dyes' fluorescence. In addition, we have heard from Dr. Steven Moorman at the University of Northern Texas that in his laboratory, he has found that glutaraldehyde fixation tends to degrade the structures of the inner ear sensory organs. Therefore, we are concerned that even if the fluorescence issue can be resolved, there is the possibility that our labeling technique will not work properly in glutaraldehyde-fixed zebrafish ears. We are therefore currently exploring the possibility of finding an alternative form of fixation which will 1) meet CSA's safety concerns, 2) not produce fluorescence which can interfere with our ability to perform the labeling experiments, and 3) provide good fixation of the structures of the inner ear.

Normal development of the vestibular system at 1-G results in an adult projection pattern of the vestibular nerve onto the vestibular nuclei, which is similar in all species of vertebrate studied. In all cases, primary vestibular afferents serving the different vestibular end-organs have distinct though overlapping patterns of axonal arborization in the vestibular nuclei. Although this adult pattern of organotopically organized projections has obvious advantages for sensory processing, little is known about 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 that neuronal activity plays in the development of the vestibular system and will thus help determine whether the lessons learned from studies of the visual system can be generalized to developmental rules for other sensory systems. In addition, these studies of the experience-dependent changes in axonal arbors in the vestibular system may reveal the anatomical substrate of the behavioral adaptation to microgravity which occurs after a few days in space. Because the vestibular system exhibits an extreme degree of evolutionary conservation, much of what is learned from the proposed experiments about the vestibular system of the fish should be applicable to the vestibular system of higher vertebrates, including humans. Whether we find that activity-dependent changes in the anatomy of primary vestibular afferents in Zebrafish raised in microgravity do occur, or whether no such changes are seen, our experiments should help to answer basic questions about the role of neuronal activity in the development of specific connections in sensory systems.

Adaptation to Linear Acceleration in Space (Atlas) - Spatial Orientation of Vestibulo-Occular Reflex and of Velocity Storage

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Funding:

Project Identification: E047
Initial Funding Date: 8/94
FY 1996 Funding: $87,655

Solicitation: 93-OLMSA-01
Expiration: 12/99
Students Funded Under Research: 2

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)
Responsible NASA Center: JSC

Task Description:

The yaw axis component of optokinetic nystagmus (OKN), optokinetic after-nystagmus (OKAN), and the vestibulo-ocular reflex (VOR) tends to align with gravity on Earth in monkeys and humans. After space flight, the yaw component of the VOR of monkeys moves toward a body rather than a gravitational frame of reference. From this it is postulated that adaptation to space causes a shift in the orientation vectors of OKN and the VOR from a gravitational to a body reference frame. How orientation is altered by introduction of linear forces in space is not known. Critical experiments are proposed in Neurolab to determine how microgravity affects the orientation vectors of the VOR and of OKN, and how they are altered by introduction of linear forces due to centrifugal acceleration in an eccentric rotator. Linear accelerations of 1-g and 0.5-g will be introduced along the subject's head interaural axis (left-ear-out or right-ear-out) and along the subject's yaw axis, inducing roll. OKN will be induced by a binocular optokinetic stimulator with subjects stationary and during centrifugation to determine whether the yaw axis component of OKN aligns with gravito-inertial acceleration (GIA), as on Earth, or with the body axis. The orientation of pursuit contributions to the OKN response will be evaluated by combining eccentric rotation with a smooth pursuit stimulus produced by movement of a small target on the screen of the binocular optokinetic stimulator. Eye movements will be recorded by a binocular three-dimensional video technique. Subjects will report their subjective motion and/or orientation sensations during centrifugation in darkness. The axes of eye rotation will be calculated using a model-based approach. Tilt in a tilt-chair will be used in ground-based testing to induce ocular counter-rolling (OCR). The astronauts will also be tested pre- and postflight during static head tilt relative to gravity. These experiments will help in

understanding how spatial orientation and OCR are altered in microgravity and in designing tasks and countermeasures in space and on re-entry.

What has been accomplished?
Centrifuge: The Training Model Centrifuge (Body Rotating Device, BRD), which was built by Aerospatiale under ESA jurisdiction is at JSC and appears to be in working order. The Flight Model is expected in March 1997. An Eye Movement Recording System (EMRS) with an attached Eye Stimulation System (ESS) is attached to the flight model. The masks for the crew and for our staff who will participate in ground-based testing are being completed. When this is completed, we should be able to get images of the eyes during rotation. The crew has not yet begun training on the rotator.

A contract was let, and plans have been developed for the Ground-Based Rotator, which will be used for our Ground-based Testing, and for the Pre- and Post-flight Testing. A final design review has taken place. The NASA IRB Safety Board asked for additional tests and verification recently, and this caused the manufacturing process to cease, while the requirements are being met. When we have NASA approval, construction will begin again. From that point, it will take about three months to finish. When it is finished, it will briefly come to Mt. Sinai before eventually being shipped to JSC. We are behind schedule in this area.

The Mount Sinai Ground-Based Rotator will have tilt capability, so that it will be possible to do ocular counter-rolling (OCR) with subjects on the centrifuge. This should save time because we will not have to move the subjects from one apparatus to another when we do the pre- and post-flight testing. The Training and Flight Rotators do not have this capability, so it will be difficult to measure OCR before we have our device.

As a result of the modification allowing us to do OCR, we will not do off-vertical axis rotation (OVAR) in the pre- and postflight testing. We reached this decision for a number of reasons: 1) We will obtain the data on OCR from static tests and during the centrifugation tests, and it is not necessary to redo them in OVAR. We will lose data on vergence, however; 2) it was difficult to move apparatus at JSC to the location where we needed it, and we did not have the personnel to do the engineering to integrate the JSC Off-Vertical Axis Rotator into our setup or to make the extra binocular three-dimensional eye movement recorder that this setup would have required; and 3) the time line was not sufficient to do all of the centrifugation tests in the pre- and post-flight crew schedule. In view of these problems, it was necessary to simplify the testing, and we decided to drop the tests using OVAR.

Eye movement recording now seems adequate from the technical point of view. The EMRS that will go on the rotator that we are building to come to Mt. Sinai is still in France and is being used to help get the Tape Processing Facility (TPF) to a functional state. The TPF is not ready yet. Great effort has gone into improving the eye images recorded by the EMRS and into the TPF that will be used to measure eye movements from the video images. Dr. Steven Moore, from our laboratory, has been traveling between New York and Grenoble to help LETI, the French company, accomplish the TPF. When the work on the TPF is close to completion, the ground-based EMRS will be shipped to Mt. Sinai for use on the Ground-Based Rotator.

We will have a Science Verification Test of the Flight Equipment from April 14th to April 19th, 1997. The flight model is coming from Europe and will include a BRD, EMRS, and ESS, along with the appropriate software to run it. We would also like to have a tilt chair that the European Space Agency has built for us, and which is part of our experimental plan, delivered at the same time. However, we have been informed that there is no room for this tilt chair at JSC, therefore testing with this chair must be postponed until after the summer.

Gilles Clement has found that Jean Loup Cretien, a French astronaut, is going to fly this summer, and that he would be willing to go through our testing before and after flight. As a result, we may have him as a subject this summer. This will give us a chance to have a preliminary test session before and after flight. This would help us practice our procedures and get preliminary data, which would be a good opportunity.

What questions have been answered?

Eye movements can be recorded in three-dimensions by binocular video techniques.

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

It lays the groundwork for the experiment by getting the apparatus in place to do the flight experiment and the pre- and post-flight testing.

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 1-G terrestrial environment of Earth. The information will also be used to direct countermeasures to overcome lags in adaptation or changes in gaze and balance due to the abnormal force field environment of microgravity. Such information and countermeasures will be critical for long-duration space flights are planned 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 spaceflight, 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. We will collect data on vergence in the present experiments. If there were problems with verging the eyes while moving toward targets after landing, it could have important 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 which will account for dynamic changes that alter the orientation of the system vectors to those of gravito-inertial acceleration. This will provide fundamental understanding of how processing of otolith information and spatial orientation are altered in the absence of gravity.

Findings from space research can readily be applied to human disorders on Earth. First, we will gain understanding of how spatial orientation is disrupted in conditions in which there is postural imbalance or gaze instability. A simple example where such information will have important clinical significance is in postural imbalance of the elderly.

We are developing a new three-dimensional, binocular video technique for recording eye movements that has great potential clinical significance. It is readily applied, non-invasive and highly accurate, and should become the method of choice for studying patients with vestibular and oculomotor disorders.

FY96 Publications, Presentations, and Other Accomplishments:

Arai, M., Dai, M., Raphan, T., and Cohen, B. (abstract) Full-field optokinetic nystagmus induced in whole body tilt positions. Vest. Res., vol. 6, no. 4S, S18 (1996).

Cohen, B. (abstract) The functional significance of the spatial orientation of optokinetic nystagmus and centrifugation. Vest. Res., vol. 6, no. 4S, S51 (1996).

Cohen, B. (abstract) Spatial orientation of the angular vestibulo-ocular reflex (aVOR): velocity storage of monkeys and humans. Vest. Res., vol. 6, no. 4S, S67 (1996).

Dai, M., Raphan, T., Kozlovskaya, I., and Cohen, B. (abstract) Modulation of ocular vergence by off-vertical yaw axis rotation in monkeys: Normal characteristics and effects of space flight. Vest. Res., vol. 6, no. 4S, S65 (1996).

Dai, M., Raphan, T., Kozlovskaya, I.B., and Cohen, B. Modulation of vergence by off-vertical yaw axis rotation in the monkey: Normal characteristics and effects of space flight. Exp. Brain Res., vol. 111, 21-29 (1996).

Highstein, S.M., Cohen, B., and Büttner-Ennever, J.A. "New directions in vestibular research" in "Annals of the New York Academy of Science." Vol. 781, 1996.

Raphan, T. and Cohen, B. "How the VOR works: Spatial orientation of the vestibulo-ocular reflex in monkey and man" in "Handbook of Clinical Neuro-Otology, Vol .1, Disorders of the Vestibular System." Edited by: Baloh, R.W. and Halmagyi, G.M. Oxford University Press, pp 20-47, 1996.

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 1996 Funding: $333,394
Students Funded Under Research: 2
Joint Agency Participation: NIH/National Institute on Aging

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: DSR (vitaport - E Net)

Task Description:

Sleep disruption is common during space flight. A survey of 58 crew members from nine space 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 crewmembers on single shift missions and 50 percent of the crewmembers 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 independently 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 FY96, through continual collaboration with the Experiment Support Scientist (ESS), Payload Project Manager, and other Sleep Team members at UCSD, NASA, and Lockheed Martin, we finalized the Training and Master Protocols, completed numerous other documentation requirements, revised the in-flight and baseline data collection (BDC) protocols, and conducted the initial crew orientation/training session.

Specifically, during the period from October to December, 1995 the Training and Master Protocols were finalized and the Experiment Document (ED) further revised. At the fourth Investigator Working Group (IWG) meeting in February 1996, we resolved several protocol issues regarding the timing of the daily administration of the melatonin/placebo pill, the timing of the daily sleep log, and constraints in the scheduling of the cognitive performance (COG) battery, among others. Subsequent to that meeting, in April, we revised and updated the Integrated Experiment Requirements Document (IERD) to reflect the latest changes to the protocol. In May, we participated in the Critical Design Review (CDR), submitting final redlines to the ED shortly after the meeting. In this same period, we produced the first version of the Software Requirements Document, detailing our plan for the development of the COG software. In August, we conducted the experiment orientation session for the Payload Specialist Candidates at Brigham and Woman's Hospital/Harvard Medical School. During this session, the candidates were provided presentations on the basics of sleep and circadian rhythms, sleep in space, melatonin, sleep recording, the experiment protocol, and complete descriptions of the methods to be used and the first instrumentation to be employed. Candidates were also provided with their first opportunity to practice the sleep instrumentation/deinstrumentation procedures and the COG battery. At the forth IWG in late August, we presented an overview of the experiment for the other attendees as part of the meeting program. Starting at the meeting and continuing thereafter, we worked with the E198 team to develop detailed revisions to the baseline data collection (BDC) scheme in order to meet guidelines set forth by JSC 22359, "Crew Scheduling Constraints. Appendix K of the Space Shuttle Crew Procedures Management Plan" while still adhering to the original experiment objectives. These revisions were then submitted by letter to NASA.

In summary, at the end of this cycle, all major documentation requirements have been met; the training has been initiated with the orientation session, and plans for subsequent training and BDC sessions are underway.

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: 10/95
Expiration: 9/99
FY 1996 Funding: $75,000
Students Funded Under Research: 3
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

Astronauts return to Earth with modestly reduced blood volumes, abnormal reductions of arterial pressure and increases of heart rate with standing, and substantial impairment of vagally mediated arterial baroreflexes. We propose studies in astronauts to test the hypotheses that 1) baroreflex malfunction after weightlessness is a consequence of neuroplasticity occurring during weightlessness and that 2) autonomic responses to acute and chronic blood volume reductions can be documented, and the mechanisms which cause such responses can be defined.

A unique aspect of this research is that muscle sympathetic nerve traffic will be measured directly in astronauts during blood volume shifts and actual head-up tilt. For the first time, muscle sympathetic nerve activity will be recorded in space. Baroreflex malfunction after weightlessness as well as autonomic responses to reduced blood volume will be investigated with controlled frequency breathing, arterial baroreceptor reflex responses, spontaneous arterial pressure and R-R interval fluctuations, lower body negative pressure, passive 60° head up tilt, ramped/graded neck pressure and suction, and valsalva maneuvers.

Subtle changes that occur at microgravity may physiologically become highly significant after return to the 1-G environment. There are compelling general scientific reasons to take advantage of the access to microgravity to study the dynamic aspects and integration of neural regulation of the cardiovascular system. The unique environment of space with the absence of hydrostatic gradients and the reduction in the overall level of physical activity drastically alters the operating conditions of the circulatory system. Analysis of the effects of microgravity on specific aspects of neural regulatory mechanisms as proposed in the present study has the potential to produce new information on properties of physiological control mechanisms.

The project matured significantly during the past year. The four investigator groups that make up the Autonomic Team worked out many important details about the integrated protocol. Details about the flight hardware were finalized as team members critically evaluated prototype flight hardware. Significant strides were also made in evaluating in-flight data processing procedures, especially software that will display critical data on in-flight screens, as well as software that will serve as stimuli for the astronaut subjects. Data acquisition and storage issues were also addressed. Significant strides were also made in crew training. Payload specialist candidates completed extensive training in microneurography at Vanderbilt University. Training also began on other procedures important to the mission. Ground-based experiments to support the mission continued, including a study to evaluate controlled frequency breathing parameters.

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

FY96 Publications, Presentations, and Other Accomplishments:

Eckberg, D.L. "Respiratory sinus arrhythmia and other cardiovascular neural periodicities" in "Regulation of Breathing, 2nd Edition, Chapter 15." Edited by: Lenfant, C., Pack, A., and Dempsey, J.A. Marcel-Dekker, New York, pp. 669-740, 1995.

Eckberg, D.L. "High and low pressure baroreflexes" in "A Primer on the Autonomic Nervous System." Edited by: Robertson, D., Low, P., and Polinsky, R. Academic Press, San Diego, 1996.

Eckberg, D.L. "Autonomic nervous system adaptation to space flight" in "Physiological Basis of Occupational Health: Stressful Environments." Edited by: Shiraki, K., and Yousef, M.K. SPB Academic Publishing, Amsterdam, 1996.

Halliwill, J.R., Taylor, J.A., and Eckberg, D.L. Impaired sympathetic vascular regulation after acute dynamic exercise. J. Physiol. vol. 495, no. 1, 279-288 (1996).

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Morillo, C.A., Wood, M.A., Eckberg, D.L., and Ellenbogen, K.A. Diagnostic utility of mechanical, pharmacological and orthostatic stimulation of the carotid sinus in patients with unexplained syncope. 45th Annual Scientific Session of the American College of Cardiology, Orlando, Florida, March 24-27, 1996.

Smith, M.L., Beightol, L.A., Fritsch-Yelle, J.M., Ellenbogen, K.A., Porter, T.R., and Eckberg, D.L. Valsalva's manuever revisited: A quantitative method yielding insights into human autonomic control. Am. J. Physiol., vol. 271, H1240-1249 (1996).

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CNS Control of Rhythms and Homeostasis During Spaceflight

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Project Identification:
Solicitation: 93-OLMSA-01
Initial Funding Date: 10/95
Expiration:
FY 1996 Funding: $25,000
Students Funded Under Research: 6
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

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

Responsible NASA Center: ARC

Task Description:

Animals have evolved and developed within the constant gravitational environment of the Earth and the dynamic changes in the environment associated with the 24-hour day. A key element in the evolution of mammals was the development of homeostasis, the ability to maintain a relatively constant internal environment. An evolutionarily older adaptation was the development of the ability of organisms to temporally coordinate their physiology and behavior both internally and with the external day. The circadian timing system (CTS) is an important temporal organizer controlling both physiology and behavior. The importance of proper CTS function is illustrated by the fact that conditions such as jet lag, shift work, and some sleep and mental disorders are frequently associated with dysfunction of the CTS. Animals exposed to the microgravity environment of space flight exhibit alterations in both CTS function and homeostasis. These alterations have included changes in body temperature regulation and metabolism, changes in the timing of physiological and behavioral functions, fragmentation of the sleep-wake cycle and even desynchronization of some rhythmic variables from the external light-dark cycle. In addition, our previous studies have shown that exposure of both mature and developing animals to hyperdynamic fields via centrifugation significantly affects both the CTS and homeostasis. This research will examine the physiology of the CTS and homeostatic control systems of animals exposed to space flight. These studies will examine the effects of space flight on four areas: (1) circadian rhythms; (2) neural responses of the circadian pacemaker and the sensory pathway for light information from the retina to the CTS; (3) adaptations in homeostatic regulation; and (4) neural changes in hypothalamic nuclei that regulate specific homeostatic functions. We will thus be examining the effects of space flight on selected physiological systems and on the central neural controllers of the same systems.

During the second year of the NASA Grant "CNS Control of Rhythms and Homeostasis during Spaceflight," several of the specific aims have been accomplished. Our studies have examined the circadian rhythms of Fischer 344 rats in an LD cycle, constant white light, and constant red light. We have determined that, as is seen in other strains, constant red light provides more robust and stable rhythms than does constant white light.

In addition, we have demonstrated that a one-hour phase-shifting light pulse will induce significant c-Fos expression within SCN neurons in Fischer 344 rats. We further tested the light pulse protocol to determine the effects of duration, intensity, and timing of a light pulse for initiating c-Fos expression in the SCN of rats in order to determine the optimal flight protocol.

We have continued to test the flight hardware so that it will be adequate for rhythm analysis. These tests have included long-term recordings from rats implanted with biotelemetry transmitters like those that will be used in the flight experiment. Our tests have allowed us to refine the surgical procedure used to implant the transmitters as well as the transmitter design. Futher analyses of the data have allowed the engineers to improve the quality of the data collection system.

We have also further refined 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 1996 Funding: $

Students Funded Under Research: 2

Joint Agency Participation: National Science Foundation

Flight Information:

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

Responsible NASA Center: ARC

Task Description:

The overall goals of the proposed research are to study the effects of microgravity on the response dynamics of the afferents of the utricle and saccule and to study any activation and action of the efferent vestibular system related to the microgravity environment. We will utilize toadfish, *Opsanus tau*, with multichannel wafer electrodes placed in the nerves innervating the saccule and utricle. These electrodes will be chronically implanted into a small cut made in these nerves, and individual axons will regenerate through the pores in the electrode to yield chronic recordings. We will record the responses of both primary afferents and central nervous system efferent fibers. We will characterize responses in normal gravity and in microgravity. Because we will record from the same fibers in both environments, we will have a measure of the effects of reduced gravity upon the performance of the otolithic organs and will assess whether the microgravity environment leads to the activation of the efferent vestibular system. Results of these experiments will bear upon theories that invoke the action of the efferent system as one of the etiologies of space adaptation syndrome. Further, studies of the cellular and systems science aspects of the vestibular system and its efferent control add information about function and may bear upon future therapies and mechanisms for the control of Earth-bound motion sickness.

We have a long-term commitment to the study of the acousticolateralis system in the toadfish, *Opsanus tau* and have studied this system extensively. Fish vestibular systems compare favorably with those of other animals. Vestibular organs, particularly the semicircular canals, were highly evolved when vertebrates first appeared; their function has not appreciably changed. Bode plots that describe canal response dynamics are remarkably similar across the vertebrate phyla. Inter-species differences appear to be related to the lifestyle of the particular animal reflecting the range of angular and linear accelerative forces experienced. Thus, we expect that our results, obtained from fish, will bear directly on the human condition.

The saccular and utricular maculae of the vestibular system primarily sense the linear acceleration vector consisting of gravitational and inertial components. We propose to chronically record otolithic afferent

responses in freely moving animals before, during, and after space flight to assess the effects of microgravity. Because this experiment will include the results of the gravitational unweighting of the otolithic mass, we should be able to delineate the effects of the inertial and gravitational components of the acceleration vector. Otolithic organ morphology and physiology has been highly conserved throughout evolution. Thus, these results should mimic the identical physiology occurring simultaneously within the ears of the astronauts accompanying our fish in the NASA shuttle. We hypothesize that there will be changes in the firing pattern of otolithic afferents when the otolithic mass is "unweighted" in microgravity; inertial responses should be unchanged.

There are profound interactions of the vestibular system with all of the body's sensory, motor, vegetative, and cognitive functions. These interactions begin with the vestibular end organ that senses the linear acceleration vector consisting of gravitational and inertial components. This information travels to the brain via the VIIIth cranial nerve to allow computations about dynamic and static position of the head. Knowledge about the variability in the function of the linear accelerometers resident in the inner ear in parallel with variations of the gravity vector will add information that has profound implications for vestibular and other bodily functions. Further, space adaptation syndrome presumably begins with "aberrant" information about the gravity vector originating within the inner ear. Those animals lacking a labyrinth do not manifest space adaptation syndrome or motion sickness. The central nervous system also contains neurons that are "efferent" or project from the brain to the labyrinth to modify incoming information before it reaches the brain. Previous extensive experiments upon the efferent vestibular system have led to the characterization of its effects upon the labyrinth. Because we will record from the same otolithic fibers in normal and in microgravity, we will have a measure of the effects of reduced gravity upon the performance of the otolithic organs and will also be able to assess whether the microgravity environment leads to the activation of the efferent vestibular system. Results of these experiments will bear upon theories that invoke the action of the efferent system as one of the etiologies of space adaptation syndrome. Results concerning space adaptation syndrome may also apply to terrestrial motion sickness.

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Anatomical Studies of Central Vestibular Adaptation

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Funding:

Project Identification: 106-30-04
Solicitation: 93-OLMSA-01

Initial Funding Date: 8/94
Expiration: 9/99

FY 1996 Funding: $119,522
Students Funded Under Research: 0

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

Flight Information:

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

Responsible NASA Center: ARC

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.

To date, six methodological studies have been conducted and completed for the Neurolab experiment. Since the experimental animals for this study cannot be perfused in space, the first study established the optimal period of time for immersion-fixation of the cerebellar tissue, and the second study determined the optimal fixation sequence for stereological and immunocytochemical studies of these immersion-fixed specimens. Based on the results of this experiment, a third study was conducted to determine the optimal fixative composition for immunocytochemical studies of immersion-fixed cerebella. The fourth methodologic study verified that the selected fixative compositon withstands the flight storage requirements. The fifth study was conducted to evaluate the impact of a mid-sagittal section through the cerebellum on the ultrastructure and chemoanatomy of cerebellar tissue. The results of this study demonstrated that the midline lesion caused major pathologic alterations in the cerebellar cortex tissue. The sixth methodological study was conducted to evaluate several strategies for quantitative analysis of cerebellar ultrastructure and immunocytochemical staining. We have now established the decision rules for tissue selection, thin-sectioning, electron microscopy, and quantitation that will be utilized for the Neurolab experiments.

The six studies above were completed in time to apply their conclusions to the Experiment Verification Test held at NASA Ames Research Center (ARC) in November, 1996. This test involved three experimental groups that were representative of the groups planned for the Neurolab mission. They included a Flight (cage-matched control) group, a Vivarium (control) group, and a Hypergravity group. To simulate hypergravity at two times against the Earth's gravitational force, this latter group was placed in the 24-ft centrifuge facility at ARC. As with the planned shuttle mission, each experimental group above included four sets of subjects: Flight Day (FD) 2 (early adaptation: N=4), FD 14 (late adaptation; N=9), recovery (R) day + 1 (early re-adaptation; N=4), and R+13 (late re-adaptation; N=7). The cerebellum from each of these 72 animals was immersion-fixed according to the protocol above and then Vibratome serial-sectioned and processed for electron microscopy. This tissue is currently being thin-sectioned, analyzed for ultrstructural tissue preservation, and further processed for post-embedding immunocytochemical studies.

This research will yield basic neuroanatomical and neurotransmitter information that will enhance current understanding of the vestibulo-cerebellum, and will clarify the role of defined cell groups and amino acid neurotransmitters in processing gravity-related information in the central nervous system. These studies will identify structural and neurochemical bases for the neuronal and synaptic plasticity that accompany CNS responses to altered gravitational environments. The studies are designed to provide insight into the morphologic and molecular changes that may occur in the brain during and follwing exposure to space flight, and to advance our understanding of the role of gravity in the maintenance of normal vestibular circuitry. Moreover, the results of these studies will contribute to our knowledge of the morphological basis for cerebellar-mediated motor learning.

Effects of Space Flight on Drosophila *Neural Development*

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93-OLMSA-01

Initial Funding Date: 8/94

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 3

Joint Agency Participation: National Science Foundation

Flight Information:

Flight Assignment: NIH-B

Responsible NASA Center: ARC

Task Description:

This project will examine the development of synaptic connectivity under the conditions of microgravity and space flight. The analysis will be confined to two motoneurons, the cells RP1 and RP3, and their three targets, muscle fibers 13, 7, and 6. More is known about the development of these two cells than for any other neurons in *Drosophila.* As a result, the RP neurons will serve as excellent benchmarks to determine whether there is any effect of microgravity on the development of individual neurons and identified synapses. Even subtle defects and targeting errors will be readily detected. In the seven abdominal segments from A1 to A7 there are paired sets of RP neurons, with each set innervating targets in the contralateral half-segment. All the sets of RP neurons behave identically. The motoneurons follow the same trajectories and choose the same segmentally homologous synaptic targets. Thus we will be able to examine synaptic development with single cell resolution in a large sample set of neurons. This will improve the accuracy of the planned morphometric characterizations. Finally, as the development of these neurons is very rapid, we can examine all the events of neural differentiation, from axon outgrowth to target exploration to the maturation of a synapse within the time constraints of a single shuttle flight. Our goals are to examine quantitatively four key events in the development and maturation of synapses during embryonic and post-embryonic life. These will be characterized using digital optical microscopy, immunocytochemistry, and single cell morphometry. As development in the *Drosophila* embryo can be suspended and resumed by temperature shifts, it will be possible to accurately control the exposure to microgravity, and examine discrete developmental exposures covering critical times in the differentiation of the motoneurons. The morphological development of RP1 and RP3 will be determined 1) as they navigate the embryonic CNS and periphery to seek 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.

General Goals and Accomplishments: The project being performed for Neurolab has moved forward excellently during the last year. We now have a stock of flies suitable for the mission, and are investigating others which will may also prove invaluable for the flight. Nevertheless, there are two outstanding elements that need to be completed. First, we wish to define the appropriate developmental stages for the study, calibrating the temperature shifts needed to expose the embryos and larvae to microgravity at specific times during development. At present, we have established the needed assays to determine appropriate times for temperature shifts. However, we currently do not have an assigned flight and mission duration so we cannot state exact times for the temperature shifts. Also we do not know the temperatures that will be available in incubators for the flight. It is important to note that we can adjust the times and numbers of shifts to best fit into the workload and schedules of the orbiter crew. As soon a flight is assigned, we will be able to promptly calibrate the exact hours when shifts will be performed.

A second issue concerns the fact that we have not yet tested any hardware for the packaging for the samples to be flown. In discussions with NASA, it has been proposed that samples be flown in BRIC-60 canisters, used for small petri dishes and capable of air exchange, but the testing and suitability of these units has not begun.

A major goal, however, has been achieved, namely to establish lines where we can assay individual neuromuscular endings directly without dissection. This was achieved by means of using the GAL4-UAS system, where we have succeeded in establishing stocks of flies where the key neuromuscular connections can be assayed directly in undissected larvae by means of the expression of endogenously fluorescent reporters in the specific motor endings. The green fluorescent protein (GFP) as a reporter allows scoring of neural anatomy en masse in whole mount using fluorescent microscopy without the need for either dissection or specific labeling. We are using the S65T mutant form, which has a dramatically brighter expression than the native protein. There are even brighter mutant forms, but UAS reporters for these are still under development in the lab and may be used if available in time for the flight.

GFP Reporter Constructs: There is no difficulty in obtaining excellent images from undissected embryos at the developmental stages when the mesoderm and nervous system are undergoing their differentiation. The problem is to identify the relevant cells conveniently, and to do so in whole mount after stage 17 of embryogenesis is a daunting task. As noted in the original proposal, we had planned to avoid dissection, as this will be a major rate-limiting step in the analysis of the embryos and larvae. A goal of the ground based studies for Neurolab is to develop robust cellular reporters to make this possible in whole mount embryos *and* larvae. We now have the tools needed to image neurons in undissected animals and get high resolution images through the cuticle in larvae. This has been made possible by the development of GFP probes of the jellyfish *Aequorea victoria* (Chalfie et al., 1994; Wang and Hazelrigg, 1994; Heim et al., 1994; Marshall et al., 1995). GFP is intensely fluorescent and shows relatively little photoinactivation.

A route to create fluorescently marked precursors is the GAL4/UAS expression system developed by Brand and Perrimon (1993). This technique allows one to use a regulatory element of interest to drive expression of the transcriptional activator GAL4. GAL4 in turn binds to UAS sequences fused to the coding region of a reporter of interest, driving expression. For our work we will use a *Drosophila* line where the regulatory UAS sequences have been fused to the coding region of GFP. We are currently focusing our efforts on a GAL4-elav driver with strong neuronal expression during embryonic and larval development. As a reporter we are using a UAS-(S65T) mutant form of GFP .

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. A second line which we are exploring makes use of expression in subsets of ventral neurons, but at present we are confident that the goals of the project can be met with the GAL4 drivers we presently have.

Drosophila strains expressing GFP in neuromuscular endings greatly simplify anatomical screens to identify neuromuscular innervation phenotypes caused by hypoactivity regimes such as microgravity. In effect, synapses can now be considered to be externally visible structures. In larvae of the C155 -GAL4/UAS-GFP stock, all of the motor endings of the SNb nerve can be clearly imaged through the cuticle in live larvae. We have found that it is possible to line up larvae on a compound fluorescent microscope using low power optics (16X 0.5NA neofluars) to obtain excellent detail of the motor endings, including the presence of the appropriate motor ending arbor types (types 1b , Is, and II), the branching patterns on the muscle fibers, as well as the presence of ectopic motor endings. These can be done both on an upright or on an inverted fluorescence microscope (either available for this project). The larvae examined in this fashion are unharmed, and will develop to adults. This makes it possible to perform screens for the effects of microgravity on synaptic structures.

A second development is the creation of a membrane-targeted version of the UAS GFP reporter, recently developed by A. Chiba at the University of Illinois, Urbana. This line will be made available to us by Dr. Chiba, and we will also cross to the elav driver. We expect that this line will have excellent labeling of fine growing tips of axons, and would be very useful for marking some of the embryonic processes.

At present, we are performing morphometric analyses on the lines to determine the degree to which they show any phenotypes due to the GAL4/UAS system or due to the expression of GFP. We are also planning to now test the effects of prolonged low-temperature (11°C) rearing on the morphology as well. Evidence to date indicates that there are no significant changes observable.

O*ther developments:* 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 have also upgraded our image capture hardware to 24bit color, and have added to our morphometric software.

a. *Benefits to space life science research:* Studies on *Drosophila* have already demonstrated that it is an excellent model system for studying synaptogenesis at the cellular and molecular level. If plans exist for long-term human exposure to reduced gravity, it is essential that all consequences to normal development and plasticity be understood at the cellular and molecular level. Vertebrate somatosensory and motor systems undergo extensive plasticity throughout life (including the adult), and therefore microgravity may potentially cause long-term changes or injury to the CNS and peripheral synapses of humans. If prolonged exposure to microgravity is anticipated (as in the case of the space station or related missions), then these studies using a model genetic system will prove valuable for identifying the kinds of changes in nervous system connectivity which may occur in humans.

b. *General benefits:* Two general benefits will result from these studies: 1. The reporter constructs will be of great value to all researchers interested in examining nervous system development in *Drosophila*, both for mutagenesis studies and for examining normal development. Thus, the *Drosophila* lines being developed specifically for the Neurolab mission will be of wide utility to the research community for other studies. 2. Insights into the role of alterations in neuromuscular activity will be of considerable value in examining the problem of synaptic plasticity.

FY96 Publications, Presentations, and Other Accomplishments:

Keshishian, H., Broadie, K., Chiba A., and Bate, M. The *Drosophila* neuromuscular junction: A model system for studying synaptic development and function. Ann. Review Neurosci. 19: 545-575. (1996).

Farrell, E., Fernandes, J., and Keshishian, H. Muscle organizers in *Drosophila*: The role of persistent larval fibers in adult flight muscle development. Devel. Biol. 176: 220-229. (1996).

Chang, T.N. and Keshishian, H. Laser ablation of *Drosophila* embryonic motoneurons causes ectopic innervation of target muscle fibers. J. Neurosci. 16: 5715-5726. (1996).

Chiba, A. and Keshishian, H. Neural pathfinding and recognition. Devel. Biol. (in press).

Fernandes, J.J. and Keshishian, H. Patterning the dorsal longitudinal flight muscles of *Drosophila*: Insights from ablation of larval scaffolds. Development (in press).

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 1996 Funding: $
Students Funded Under Research: 2

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)
Responsible NASA Center: ARC
Flight Hardware Required: RAHF

Task Description:

The proper development of the nervous system requires sensory input. For example, the development of sight requires visual input during a critical period. Children whose eye muscles are not properly aligned, a common condition called strabismus, tend to suppress vision in one eye. If the one waits beyond the critical period, even after the eyes are re-aligned, vision may not be restored. Visual input during the critical period is required for a person to see normally. This study is designed to determine whether the sensory information provided by gravity after birth is necesary for the development of spatial ability. Our first step toward answering this question will be to study the structure and function of brain areas, particularly the hippocampus, involved in spatial memory. The number of synapses will be counted in rats returning from space and compared to ground-based controls. The expression of certain key molecules that appear in the mature brain will be measured. We will determine whether several neurotransmitter systems, cytoskeletal proteins, and synaptic proteins are altered in their distribution. Learning how the brain handles gravitational cues allows us to begin to assess the feasibility of long term habitation in space.

To this point, we have developed the conditions for fixing material to optimize data acquisition and coordinate our tissue requirements with those of the other members of the mammalian development team. The electron micrographs provide sufficient resolution to quantitate synapses. The antibody labeling with MAP2 was able to visualize the dendritic architecture in the hippocampus and antibodies to glutatmate receptor subtypes demonstrate unique developmental patterns that will permit us to detect alterations under conditions of space flight.

An enhanced understanding of early brain development is crucial to providing infants and children an environment which allows the brain to attain its maximum capacity. This project will provide insights into early brain development. Applications to current pressing medical conditions are also expected because spatial ability is frequently affected in a variety of brain diseases, including Alzheimer's disease and stroke.

Ensemble Neural Coding of Place and Direction in Zero-G

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Funding:

Project Identification:

Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94

Expiration: 11/99

FY 1996 Funding: $213,786

Students Funded Under Research: 5

Joint Agency Participation: NIH and Office of Naval Research

Flight Information:

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

Responsible NASA Center: ARC

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.

This year was devoted to the development and testing of the experiment hardware and other flight equipment: (1) A biocompatibility test of the Research Animal Holding Facility (RAHF), in which implanted rodents were housed in flight-like RAHF units, confirmed that the hyperdrives were stable and protected. However, the rodents had difficulty acccessing food in the cage, and a number of manipulations were made to improve their access to the food; (2) redesigned hyperdrive implants were tested and were found to work very well; (3) various configurations of a Temporary Holding Unit to house the animals during the experiments in the General Purpose Work Station (GPWS) were tested, and the final design of a Rodent Sleeping Pouch, made of a stiff but flexible fabric, was adopted as the easiest solution to hold the animals comfortably and to allow access for electrode manipulations; (4) because of the large number of animals that need to be trained in parallel, we developed a

computer-automated training facility that allows 4 rats to be trained simultaneously; (5) electrodes, hyperdrives, and other support equipment were prepared to support the Experiment Verification Test (EVT) at Ames Research Center in the Fall of 1996; and (6) the development of the Data Acquisition System hardware and software continued during this period, and a prototype was built to support the EVT.

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.

Glial Cell Reaction from Space Flight

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Funding:

Project Identification: BSP-008
Initial Funding Date: 4/95
FY 1996 Funding: $26,286

Solicitation: 93-OLMSA-02
Expiration: 3/96
Students Funded Under Research: 0

Flight Information:

Responsible NASA Center: ARC

Task Description:

This is a project submitted in response to NRA 93-OLMSA-02 "Biological Flight Experiments Tissue Sharing Proposal." The goal of the project is to determine the effects of space flight on glial cell proliferation and reactive gliosis in the adult nervous system.

Gliosis and glial cell proliferation are a common reaction to injury of the CNS. The goal of this experiment is to determine if space flight, notably lift-off, produces subclinical damage to the brain, presumably as a result of compression by the forces associated with launch.

Brains from rats used in the Physiological Anatomical Rodent Experiment 3 (PARE.03) were requested through the NASA tissue sharing program. These animals had received subcutaneous injections of tritiated thymidine (1 uCi/g) at R+0, R+24, and R+72; the brain had been removed and stored frozen as part of the tissue sharing program.

A total of 18 brains (9 flight and 9 control) were received. Unfortunately, these brains had been badly damaged during the initial dissection and freezing and their condition on receipt in this laboratory was poor; the best description of the brains is that they were flattened so that they resemble quarters. This has made processing difficult because achieving the goals of this project requires that we be able to identify anatomical landmarks in the brain. These landmarks have been completely distorted. Nevertheless, we have determined using one of the control brains that we can successfully detect the radioactivity in the brains. Therefore, at this time we have continued to hold the brains in frozen storage as we attempt to replicate the poor condition of these brains on locally obtained specimens. Once we have done this we will be able to return to the tissue obtained from the PARE.03 flight and evaluate the brains acording to the original goals of the project.

Gliosis is a common reaction to injury to the CNS; however, it is not clear whether gliosis is beneficial or deleterious to the recovery of function after injury. The possibility that gliosis might occur during space flight, i.e., either during lift-off or during weightlessness, provides a unique opportunity. The classic view of gliosis is that glial cell proliferation after CNS injury antagonizes would healing by formation of a glial scar. From the

perspective of the space program it is interesting to know if gliosis ocurs because there are no clinically detectable signs of injury.

From the perspective of Earth benefits these experiments are relevant because they may provide a useful way to assess the role of glial cell proliferation and gliosis in conditions on Earth (e.g., mild head trauma) in which no clinically detectable signs are apparent, but in which an injury has obviously been inflicted. In addition, if the gliosis in "space flight" is due to launch, then a hypergravity launch profile may be a useful and reproducible paradigm for the study of mild head trauma.

Reduced Gravity: Effects in the Developing Nervous System

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Funding:

Project Identification:

Solicitation: 93-OLMSA-01

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 0

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

Flight Information:

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

Responsible NASA Center: ARC

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); and 2) neuronal migration (i.e., the rate of movement and attainment of proper position. 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. 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. No multigeneration studies are planned. Ideally, experiments will be performed on mice for which there already is a great deal of data from other NIH supported projects.

This is one of the component projects of Neurolab. The specific aims of this project remain the same as they were in the "modified integrated proposal." There are two specific aims:

1. to determine the effects of space flight on cell proliferation in the developing cerebral cortex, and

2. to determine the effects of space flight on neuronal migration in the developing cerebral cortex.

Both of these specific aims will be performed in mouse embryos. Both also require the development of mathematical model and computer simulations from our current Earth-bound research. The first specific aim will also be performed in the developing cerebellum of rat pups.

A third Specific Aim of the original proposal which was to study neuronal differentiation in the developing brain was eliminated during the "Definition Phase" of this project because of the impossibility of having live births on orbit, i.e., in the space shuttle. However, this third aim remains "alive" in the planning process as a contingency in the remote event of a need to land the Neurolab after only a few days on orbit.

The current scheduled date for the launch of the Neurolab mission is March 19, 1998.

Preliminary results to date have revolved around: 1) modifications of our established protocols in order for them to be successfully applied in the space shuttle; 2) the continued development of our mathematical models and computer simulations; and 3) the collection of preliminary and control data in the strains of mice and rats to be used on Neurolab. All of these preliminary studies are being completed on time and we are on schedule for the development of the project.

In the past year, we have completed several studies at NASA Ames' request with regard to compatibility of fixatives and solutions between our science needs and flight conditions. These have focused on storage conditions, i.e., time and temperature tolerances, etc. In addition, we have developed and tested specific criteria to optimize the selection of pregnant mice for flight. The criteria that we have develped assure that the flight experiments will be successful.

The "Experimental Verification Test" (EVT) for the Neurolab flight was performed in October- November 1996. This included will be a complete dry-run of Ames portion of the Neurolab flight. Hypergravity experiments were conducted as part of the EVT. This is an essential control for the effects of microgravity. The prediction is the "macrogravity" (2-G) will have the opposite effect on cell cycle kinetics that microgravity will have. EVT itself went well and all portions of the planned experiments were completed successfully. Tissue from EVT is being analyzed. No data is yet available.

The mathematical models and computer simulation portion of the project have been proceeding extremely well. We have had several publications that have been supported by these funds. These mathematical models will enable the data obtained in flight, from ground controls and from hypergravity experiments to be interpreted in a quantitatively precise conceptual framework. In addition, the papers provide evidence of the significance beyond the space benefits of this project.

The effects of space flight on the developing CNS are essentially unknown. Our "null hypothesis" is that microgravity will have a profound effect on cell proliferation because of the loss of bouyancy of the organelles which will disrupt intercellular mechanisms associated with cytoskeleton and with energy utilization required during mitosis. This is of general significance to space flight because cell proliferation also occurs in adults, including humans, chiefly in the skin, gut, and immune systems. It is also of relevance to wound healing, etc., both in space and on Earth. The data to be collected will provide specific and new insight into the complex cellular processes associated with cell proliferation and the intracellular mechanisms that regulate and control this process. Since these events occur on Earth in every multicellular organism, these experiments are of general relevance to an understanding of the basic biological process of cell proliferations. Thus, the results that we obtain will be of significance in understanding the normal controls on the regulation of cell proliferation and cell number during development, in cancer, in immune system function, in wound healing, etc.

We plan to continue our studies of normal development and the development of our mathematical models and computer simulations. These studies are of signficant value in their own right and also serve to maximize the scientific return and interpretability of the animals from the Neurolab flight.

FY96 Publications, Presentations, and Other Accomplishments:

Takahashi, T., Nowakowski, R.S., and Caviness, Jr., V.S. Interkinetic and migratory behavior of a cohort of neocortical neurons arising in the early embryonic murine cerebral wall. J. Neurosci., 16, 5762-5772 (1996).

Takahashi, T., Nowakowski, R.S., and Caviness, Jr., V.S. The mathematics of neocortical neuronogenesis. Exp. Neurol., 137, 357-366 (1996).

Takahasi, T., Nowakowski, R.S., and Caviness, Jr., V.S. The leaving or Q fraction of the murine cerebral proliferative epithelium: A general model of neocortical neuronogenesis. J. Neurosci., 16, 6183-6196 (1996).

Role of Visual Cues in Spatial Orientation

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Funding:

Project Identification: E136
Solicitation: 93 OLMSA-01
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 2

Flight Information:

Flight Assignment: Neurolab (STS-90, 3/98)
Responsible NASA Center: JSC

Task Description:

The goal of this Neurolab experiment is to better understand how humans transform spatial orientation cues from egocentric to exocentric frames of reference, so as to perceive linear and angular orientation ("tilt," "location," "direction") and linear and angular motion ("speed" and "rotation"). On Earth, gravity provides an omnipresent cue which anchors our exocentric reference frame. Perceived self-tilt influences how we recognize objects around us and judge their angular orientation and shape. Conversely, the tilt, direction, motion, and shape of objects influence our own self-tilt, -direction, and -rotation. Perception of self orientation and object orientation are thus interdependent. In orbit, as we move in three dimensions, to what extent are we able to maintain a consistent exocentric reference frame? Does our ability to recognize object orientation and shape depend on this? How does the orientation, shape, and motion of objects around us influence self-orientation? What is the influence of haptic cues and otolith unweighting? Astronauts often experience striking, labile "visual reorientation illusions" 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 three 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 object, and to define how altered CNS gravireceptor cue weighting influences the onset of visually induced linear motion sensation. Pre- and post-flight controls are required. The tests measure: 1) the influence of scene symmetry, scene rotation, orientation expectation and haptic cues on self-tilt; 2) the effect of perceived orientation on visual object recognition and shape perception; and 3) the onset of x-axis

illusory linear self-motion ("looming 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 experiment and the Virtual Environment Generator (VEG) design both passed through a NASA critical design review in November 1996. The bulk of the experiment manager software was written in preparation for the first crew-training session that occurred mid-December 1996. VEG software development system was delivered to MIT. Since then, the PI has been cooperating with the LMES VEG team to finish the development of the experiment manager software, the supporting session manager, and the archive manager software components. The PI has also been cooperating with mission management on timeline development. Due in part to vendor delivery delays, the PI has not received main pieces of VEG flight hardware, namely, the helmet-mounted display, the head/hand position tracker, and the subject restraint system components. Therefore, many supporting science development activities, including hands-on crew training and KC-135 0-G experiments, have been delayed by four months as of April, 1997. Hardware delivery is anticipated soon, however, so we expect the project to be nearly back on schedule by mid-summer, barring unforeseen technical problems.

Many people are familiar with the illusions of visually induced self-tilt, circular-vector and linear-vector through personal experiences in IMAX and "Circle Vision" theaters, amusement park rides (e.g., Disney's "Star Tours" and Universal's "Back to the Future"), or new "virtual reality" entertainment systems. There is currently considerable interest in using helmet-mounted "virtual reality" display techniques in a wide variety of applications in surgery, architecture, arts, education, manufacturing, mining, etc. Results from the Neural studies of interaction between visual, vestibular, and proprioceptive orientation cues in zero-G are generically applicable to the design of night simulator and "virtual reality" vision, motion, and cueing systems. Users of many existing systems report difficulty maintaining a consistent spatial frame of reference and motion sickness, because insufficient attention has been paid to providing appropriately matched visual, vestibular, and proprioceptive orientation cues. 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 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 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:

Project Identification: Solicitation: 93 OLMSA-01

Initial Funding Date: 8/94 Expiration: 11/99

FY 1996 Funding: $35,853 Students Funded Under Research: 6

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

Flight Information:

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

Responsible NASA Center: ARC

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.

Hamiltion microsyringes and injectors have been identfied as appropriate means of delivering 5-10 microliters of retrograde tracer to muscles in 0.5 microliter aliquots. The retrograde tracer labels soleus and EDL motor neurons 2 days after injection. This inflight capability for quick freezing was not developed for this mission. Tissues will be mildly fixed and used for a reduced number of immunostaining procedures to assess fiber type differentiation and neuromuscular junction maturation. An inflight procedure for whole body fixation of neonatal rats has been developd for spinal cord preservation. Fixation is less than laboratory standard and further attempts are underway to improve the process. The time for performing the inflight perfusion is also being shortened in order to accomplish a sample size commensurate with robust statitistics.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Riley, D.A. Inflight and postflight changes in skeletal muscles of rats flown in NASA Spacelabs and Cosmos Biosatellites. COSPAR Abstracts, (1996).

Riley, D.A., Ellis, S., Slocum, G.R., Sedlak, F.R., Bain. J.L.W., Krippendorf, B.B., Lehman, C.T., Macias, M.Y., Thompson, J.L., Vijayan, K., and DeBruin, J.A. Inflight and postlfight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. J. Appl. Physiol., 81, 133-144 (1996).

Flight Verification Test of Nursing Facility

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Funding:

Project Identification:

Solicitation: AO-93-OLMSA-01

Initial Funding Date: 8/94

Expiration: 1/97

FY 1996 Funding: $75,000

Students Funded Under Research: 5

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R3 (STS-72, 11/95)

Responsible NASA Center: ARC

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 were processed 8 months postflight to determine whether long-term changes were induced. Preliminary results indicate that the soleus muscle/body weight ratios of flight rats are less than normal, whereas the EDL ratio is normal. This indicates that space flight selectively retarded growth of the antigravity muscle. The maturation of the soleus motor nerve terminals

was also delayed or aberrant where those of the EDL were normally developed. Finally, muscle fiber type differentiation appears altered in the soleus. Further analyses are in progress, but it appears that microgravity has a dramatic negative influence on the development of the neuromuscular system of antigravity muscles.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Riley, D.A. Inflight and postflight changes in skeletal muscles of rats flown in NASA Spacelabs and Cosmos Biosatellites. COSPAR Abstracts, (1996).

Riley, D.A., Ellis, S., Slocum, G.R., Sedlak, F.R., Bain, J.L.W., Krippendorf, B.B., Lehman, C.T., Macias, M.Y., Thompson, J.L., Vijayan, K., and DeBruin, J.A. Inflight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. J. Appl. Physiol., 81, 133-144 (1996).

Autonomic Neurophysiology in Microgravity

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Project Identification: E095
Solicitation: 93-OLMSA-01
Initial Funding Date: 10/94
Expiration: 9/99
FY 1996 Funding: $173,000
Students Funded Under Research: 9
Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

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

Responsible NASA Center: JSC

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.

In ground-based evaluations of the LBNP chamber originally developed by Friedhelm Baisch, we have confirmed that all aspects of our experiment are able to be carried out successfully using this equipment and can be done within our working time frame.

A major task during the past year has been the training of the payload specialist candidates in the technique if microneurography. For this purpose, the four payload specialists spent more than two months in Nashville this past summer gaining experience in this technique. Each of the payload specialists picked up the technique, and each had been successful in microneurography within two or three days of arrival. During the subsequent weeks, they gained considerable experience at this, and by the end of the two-month period, all payload specialists had reached the targeted level of proficiency and had conducted many dozens of successful microneurograph readings. We consider the training sessions to be a notable success from every standpoint. In early January 1997, a final ground-based study of feasibility was carried out at Vanderbilt with the participation of investigators from Southwestern, Richmond, and DRL. This study incorporated as many of the equipment items that will fly aboard the Neurolab mission as possible. This study was a complete success.

The results of these studies will improve our understanding of autonomic mechanisms that regulate blood pressure. We hope that this will be translated in the development of improved countermeasures to alleviate the orthostatic symptoms astronauts experience upon return to Earth. It should be noted that Orthostatic Intolerance is the most common autonomic abnormality that affects a substantial number of patients. These patients are usually young and otherwise normal, but are significantly disabled by their inability to remain upright because of symptoms of cerebral hypoperfusion. This disorder is poorly understood, therefore treatment remains inadequate. We believe the knowledge gained by the Neurolab experiments will help improve the treatment of these patients.

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Multidisciplinary Studies of Neural Plasticity in Space

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Funding:

Project Identification: 1 U01 NS33448-01
Solicitation: 93 OLMSA-01
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 2
Joint Agency Participation: NIH/National Institute of Neurologic Disorders and Stroke

Flight Information:

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

Responsible NASA Center: ARC

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 microgravity 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.

During FY96 sufficient data were collected to conclude statistical analysis of synaptic ribbon changes in hair cells of gravity sensors of rats exposed to microgravity on the SLS-2 mission. More than 1,000 hair cells and over 6,500 synaptic ribbons were studied in 12 samples. In all, synaptic ribbons in 1,200 serial sections were recorded. The results have been written into a report for publication in FY97. The main finding is that type II

cells are particularly affected by space flight. Type II hair cells may be detectors of gravity and type D reconstructions were made from the serial sections to illustrate particular facts about the neuronal connections in gravity sensors. The reconstructions showed that type II hair cellls are arranged in small clusters, typically of three cells, with the surrounding calcyes providing overlapping innervations to the type II cells. In one reconstruction, five calyces and one afferent nerve fiber together provided 18 processes to one type II cell. The findings continue to support the concept that type II cells are integrated into the same neuronal circuitry that supplies type I cells. However, the ultrastructural research also showed that type II cells receive presynaptic processes of afferents as well as postsynaptic processes, and that some synaptic interactions are reciprocal. This result has led to the concept that there are local microcircuits in gravity sensors, and that type II cells are inserted into these microcircuits. This fact can help account for the finding that type II hair cells are particularly affected by space flight, since local circuits help shape a response in other systems. In this case, gravity-sensitive cells would be interacting with the output of type I cells to shape the neuronal responses to transient linear accelerations. These new insights provide a basis for research into the molecular events underlying synaptogenesis and deletion in gravity sensors. They also give new emphasis to the need to study the development of these interesting endorgans in microgavity on the space station, to learn whether neuronal connectivities will be altered to such an extent during development that readaptation to Earth's 1-G will be problematical.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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The Stress of Space Flight: Effects on Learning

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Funding:

Project Identification: E052
Initial Funding Date: 12/95
FY 1996 Funding: $177,500

Solicitation: 93-OLMSA-01
Expiration: 12/99
Students Funded Under Research: 6

Flight Information:

Flight Assignment: NIH-H (STS-95, 1998 [target])
Responsible NASA Center: JSC

Task Description:

From lift-off to post-flight re-acclimation, space flight is clearly a tremendous stressor. In space, astronauts are required to perform complex physical and mental tasks, yet relatively little information has been gathered on how this unique stressor impacts on the basic components of learning and performance. We propose to study how prolonged exposure to microgravity affects nonassociative and associative learning.

Nonassociative learning will be assessed by measuring sensory reactivity (startle response) to sudden noise. Through the concomitant measure of heart rate spectrum (HRS) and eyelid electromyography (EMG), we will assess sensory reactivity to white noise stimuli of various intensities. Associative learning guides the allocation of neural resources and provides a framework for the acquisition of casual relations. Classical conditioning of the eyeblink response provides a convenient platform on which to observe the acquisition of these relations. We have proposed to study the effects of space flight and adaptation to microgravity on the acquisition of this conditioned response using a 2-tone discrimination paradigm. As with nonassociative learning, our goals in the present proposal are to expand our ground-based subject pool and to perform more extensive 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.

The project team met in Houston in July 1996, for a Preliminary Design Review (PDR) of the E052 project.

Tests of the E052 hardware prototype during FY96 indicated that changes in the acoustic stimuli were required. A second hardware prototype was developed with E052 project engineers to deliver the stimuli required for eyeblink conditioning. Further operational testing of the apparatus will be conducted in FY97.

These studies directly examine the interplay between environmental stressors and adaptation. Rarely does the scientist interested in the psychophysiological aspects of stressor exposure have the opportunity to measure human reactivity during a naturally occurring sequence of stressors. Moreover, the stressors of space flight and adaptation to microgravity have the potential of being more homogeneous in terms of intensity between individuals. Since stressor intensity is considered a critical variable in the genesis of stress-related mental illnesses (such as post-traumatic stress disorder), the results of these studies could indicate how stressor intensity contributes to these disease processes.

FY96 Publications, Presentations, and Other Accomplishments:

Servatius, R.J., Shors, T.S., Peterson, R., Amberboy, C., Grounds, D., and Tapp, W.N. (abstract) Where no Pavlovian learning paradigm has gone before. Eyeblink conditioning in space. Soc. Neurosci. Abst., vol. 22, 1648 (1996).

Effects of Microgravity on Postnatal Motor Development

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Funding:

Project Identification: Solicitation: 93 OLMSA-01

Initial Funding Date: Expiration:

FY 1996 Funding: $ Students Funded Under Research: 1

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

Flight Information:

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

Responsible NASA Center: ARC

Task Description:

The objective of this proposal is to evaluate the adaptability of the motor nervous system to environmental demands. The force of gravity is one of the few constant factors during the evolution of the nervous system and, for this reason, is deeply embedded in its functioning. This is particularly marked for the motor system since an animal's posture is dependent on the appropriate force being maintained at every joint of the articulated skeleton to oppose the action of gravity. The experiments examine the adaptability of the motor system to changes in gravity and the mechanisms underlying such neuronal plasticity. Since young animals are particularly susceptible to changes in their environment, they offer a sensitive model for nervous system plasticity. Our working hypothesis is that: 1) a normal gravitation field is essential for the normal postnatal development of the motor system; 2) elimination of weight-bearing will lead to profound changes in motor system organization; 3) changes in motor function will be most marked when animals are exposed to microgravity during "sensitive periods," and 4) functional changes will persist into adulthood when animals are exposed during "critical periods" of motor development. We will use behavioral, electromyographic (EMG), and molecular approaches to study rat pups from postnatal day 6 (P6) through P31 in ground and flight studies. Behavioral measures will evaluate the development of interlimb coordination (e.g., swimming and walking), dynamic postural stability (e.g., placing reactions and righting reflexes), and complex motor skill (e.g., rope, ladder, and rod climbing). EMG recordings of activity from major hindlimb muscles will be combined with video-based motion analysis of treadmill walking to examine the neuronal basis for locomotion in control and experimental animals. Biochemical and immunohistological studies will determine the pattern of expression of glutamate receptor subunits genes in the lumbar spinal cord. Pre-flight ground experiments will study animals reared under conditions of simulated microgravity (tail-suspension), hypergravity (centrifugation), and a simulated shuttle mission gravitation profile, hypergravity-microgravity-hypergravity. Rat pups will be reared aboard the shuttle P7-P21 (sensitive period) and P17-31 (critical period). In-flight experiments will evaluate placing reaction, complex motor skills, and vestibular reflexes. Post-flight, we will study the ability of the animals to adapt to the relative hypergravity of Earth. Neurolab offers a unique opportunity; the flight rats will be the first mammals to have developed the

majority of their motor skills under conditions of microgravity. Study of these "space rats" will further our understanding of the role of gravity in postnatal development and to what extent the nervous system is able to adapt to changes in gravity. Since the major elements of the motor system—neurons, muscles, and bone—will develop under condition of microgravity, these experiments will also further our understanding of the plasticity and interaction of these systems during postnatal development and motor function.

We have been working with ARC to develop and test experimental unique hardware for in-flight testing of motor performance. Through this process, the in-flight protocol and hardware has been modified and it is close to completion. During the Experiment Verification Test (EVT) we found that unfixed tissue can successfully be preserved for light microscopy using tissue slicing and microwave procedures. However, the technique needs to be refined to improve preservation of brain tissue for electron microscopic studies. As part of the EVT, we carried out a 16-day hypergravity study. This was the first time that rat neonates had been exposed to 2-G. We found that, as in adult animals, it took three days for the neonates to adapt to the hypergravity environment. A difference was seen in the motor performance of the centrifuged and control animals. However, unlike exposure to microgravity or simulated microgravity, these changes were not long lasting. Further analysis of data from the animals on the NIH-R3 flight (STS-72) has lead to change in our post-flight protocols for Neurolab.

The results of such a study will further our understanding of postnatal neuronal development, as changes in gravity provide an excellent noninvasive model for investigating nervous system plasticity. Mechanisms that underlie neuronal development are often the same that regulate plasticity and repair in the adult nervous system. For example, axotomized adult motoneurons show many properties of immature motoneurons; polyinnervation typical of the early postnatal period is seen after sciatic nerve block within adult motoneurons. Recently, it has been shown that activity-dependent synaptic plasticity in the adult and young animals follow the same general principles. Insights gained from space may be applicable to a number of neurologic conditions when plasticity of neuromuscular function would be desirable. For example, if reorganization within the nervous system could be enhanced by manipulating the glutamate receptor phenotype of neurons, enhanced motor function could result after trauma to nerve, muscle, or spinal cord, or in degenerative conditions of the neuromuscular system. Simulated weightless paradigms may also be relevant to pediatric cases where children are confined to bedrest.

Flight Verification Test of Nursing Facility

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: AO-93-OLMSA-01

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-R3 (STS-72, 11/95)

Responsible NASA Center: ARC

Task Description:

No additional information was supplied by the principal investigator.

Sleep and Respiration in Microgravity

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Funding:

Project Identification: E198
Solicitation: 93-OLMSA-01
Initial Funding Date: 10/94
Expiration:
FY 1996 Funding: $550,000
Students Funded Under Research: 3
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Flight Information:

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

Responsible NASA Center: JSC

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 μg. In many people, nocturnal hypoventilation leads to hypoxemia, and hypercapnia, and is a potent arousal stimulus. During sleep, many people experience periodic breathing, and there is one report of sleep apnea actually occurring in flight aboard the Russian Space Station Mir. Possible changes in the chemoreceptive control of ventilation brought about by exposure to μg may well contribute to alterations in the sleep pattern in μg.

We will measure respiration during sleep in mg by instrumenting subjects with a Respiratory Inductance Plethysmograph (RIP) and pulse oximeter allowing continuous measurement of the motion of both the rib cage and abdomen and arterial oxygen saturation. In addition, subjects will be fitted with an EEG, EOG, an ingestible body temperature sensor allowing us to determine sleep stage, and an ECG. From these sensors, we can determine changes in ventilation, relative rib cage and abdominal contribution to ventilation, thoraco-abdominal 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 μg, and we expect to find that there will also be changes in the neurological control of ventilation in μg. We expect that these will manifest themselves as changes in the pattern of sleep.

In addition, we will study the neurological control of ventilation by measuring the ventilators response to both hypoxia and hypercapnia. Inflight, we will measure the quasi-isocapnic hypoxia response and the hypercapnic rebreathing response. In addition, we will measure cardiac output, diffusing capacity lung water, and resting oxygen consumption. These will be supplemented by RIP and pulse-oximetry measurements allowing

determination of respiratory timing without the interference of a mouthpiece and arterial oxygen saturation. 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 μg will shed considerable light on the changes in the neurologic control of ventilation that occur when gravity is removed.

In FY96, we progressed from PDR stage to early crew training. The first hypoxic scrubber was delivered to UCSD, and Crew Orientation Training was conducted at UCSD in Set 1996. A final team selection of the choice of digital sleep recorder was made and a test unit was procured. After extensive testing, an order has been placed for the flight units. Early Neurolab flight software development was begun. The next major milestone will be Science Verification Testing scheduled for March 1997.

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.

Development of Vestibular Organs in Microgravity

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Funding:

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Solicitation: 93 OLMSA-01
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 0
Joint Agency Participation: National Science Foundation

Flight Information:

Flight Assignment: CEBAS (STS-89, 1/98) and Neurolab (STS-90, 3/98)
Responsible NASA Center: ARC

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.

The fine structure and development of the statocyst in *Biomphalaria glabrata* have been elucidated. Unlike *Aplysia*, the statocyst of *Biomphalaria* has only statoconia, even in embryonic animals before they hatch from the spawn pack, whereas *Aplysia* has a single statolith at larval stages. This correlates with the fact that *Biomphalaria* never go through a stage in which the animal swims freely in open water, but rather crawl as soon

as they leave the spawn pack. We have also shown that the statoconia grow after they have been exocytosed into the statocyst lumen. This suggests that, even in the cyst lumen, the statoconia receive additional organic material which stimulates further mineralization. This material must be generated either in the statocyst receptor cells or the supporting cells, where the statoconia are originally formed. This would provide a mechanism by which statoconial mass could be regulated in an altered-gravity condition.

Crawling paths have been videotaped and quantitatively assessed in a large number of adult and 2-mm juvenile snails of both pigmented and albino strains of the snail. All animals preferentially crawl in a downward direction. The larger animals crawl at a greater rate and with a more consistent direction (the dispersion is less in the vectors describing the paths in older animals). Similarly, newly hatched snails preferentially swim downward. We have also studied primarily 2-mm snails (the largest size we would expect from animals conceived in flight) on the rotating table. These animals preferentially crawl in the direction of the artificial "gravity" created by the centrifugal force. Thus, if animals conceived and developed in flight do not show preferential crawling directed to gravity after their first introduction to gravity at Return, we will be able to test their "threshold" for such behavior by imposing higher than normal 1-G forces, using the centrifuge.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Pedrozo, H.A., Schwartz, Z., Luther, M., Dean, D.D., Boyan, B.D., and Wiederhold, M.L. Mechanisms of adaptation to hypergravity in the statocyst of *Aplysia californica.* Hearing Res., 102, 51-62 (1996).

Steyger, P.S. and Wiederhold, M.L. Visualization of newt aragonitic otoconial matrices using transmision electron microscopy. Hearing Res, 92 (1/2), 184-191 (1996).

Inflight Radiation Measurements

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Funding:

Project Identification: 5.2.1
Solicitation: US/RSA Negotiations
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

Additional information was not available in time for publication.

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

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Funding:

Project Identification: 4.2.4
Solicitation: US/RSA Negotiations
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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 (CNS) to permit new sensory-motor strategies to emerge in the novel sensory environment of microgravity. However, the adaptive state achieved on orbit is inappropriate for a 1-G environment leading to postural and gait instabilities 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 2 m 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.

Summary of Progress

1) Mir-18 pre- and postflight data were collected on three subjects. Three preflight (120, 45, and 10 days before launch) and six postflight (1, 4, 6 , 9, 12, and 180 days after landing) data collections were performed. Two preflight and the last postflight collection took place at the Gagarin Cosmonaut Training Center (GCTC), Star City, Russia. All other sessions were collected at the Johnson Space Center (JSC).
2) Mir-19 pre- and postflight data were collected on two subjects. Three preflight (120, 45, and 10 days before launch) and six (1, 4, 6 , 9, 12, and 180 days after landing) postflight data collections were performed. One preflight collection took place at JSC; all other sessions were collected at GCTC.

Data Analysis Summary:

All preflight and two postflight video data sessions have been processed through the following steps of data reduction:

Data Analysis Process

1. Video Data Reduction

1) Tracking: calculating 3-dimensional trajectories of each body-fixed marker using the computer-based algorithms and camera calibration procedures.
2) File Transfer: converting data to analyzable MATLAB format and transferring the 3-D trajectory output files and electrooculography and footswitch data to a computer network. This includes renaming trajectory variables and synchronizing EOG and footswitch analog data with trajectory traces.

These are preliminary steps that are required before further data analyses can be performed.

2. Video Kinematic Analysis

X, Y, Z movement trajectories were obtained from markers placed on the head, torso, and legs. The following parameters will be derived:
• Coherence between trunk movement and compensatory head movements.
• Amplitude of the predominant frequency of pitch, yaw, and roll head movements obtained through Fourier Analysis.
• Phase plane analysis of lower limb kinematics.

3. Electromyographic (EMG) Analysis Of Leg Muscle Activation Patterns

EMG was collected bilaterally from the following muscles: tibialis anterior, gastrocnemius, rectus femoris, and biceps femoris. The following parameters will be derived:
• Pearson R correlations between normalized pre- and postflight waveforms.
• Coefficient of variation (i.e. standard deviation divided by the mean amplitude) around both heel strike and toe-off phases of locomotion.

4. Eye Movement Analysis

Electrooculography (EOG) was used to collect compensatory horizontal and vertical eye movement during locomotion. The following parameters will be derived:
• Coherence between trunk movement and compensatory eye movements.
• Phase relationships between eye, head, and trunk movements.

Preliminary observations showed:
1) Crew members show significant alterations in head-trunk coordination following long-duration space flight.
2) Crew members showed disruption in lower limb muscle activation patterns during locomotion that exceeds that shown by shuttle crew members.
3) Microgravity-induced alterations in head-trunk coordination during locomotion may play a central role in astronaut locomotor dysfunction that occurs following space flight.

These data helped to expand and focus a second study that is part of the NASA-Mir program. Data from Mir-18 and Mir-19 subjects will be combined with those obtained in the NASA-Mir program.

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, and postural and locomotor control. These investigations are aimed at determining the magnitude and time constants of adaptation to microgravity and readaptation to Earth gravity as a function of space flight mission duration.

Performing this investigation following extended stays on 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.

FY96 Publications, Presentations, and Other Accomplishments:

Bloomberg, J.J., Reschke, M.F., Huebner, W.P., Peters, B.T., and Smith, S.L. Locomotor head-trunk coordination strategies following space flight. J. Vestib. Res., (in press).

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

LaFortune, M.A., McDonald, P.V., Layne, C.S., and Bloomberg, J.J. Space flight modifications of the human body shock wave transmission properties. Annual Meeting of the Canadian Society for Biomechanics, Vancouver, B.C., Canada, August, 1996.

McDonald, P.V., Basdogan, C., Bloomberg, J.J., and Layne, C.S. Lower limb kinematics during treadmill walking after space flight: Implications for gaze stabilization. Exp. Brain Res., 112, 325-334 (1996).

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

McDonald, P.V., Lafortune, M.A., Layne, C.S., and Bloomberg, J.J. Challenges to head stability after space flight. Society for Neuroscience Annual Meeting, November, 1996.

McDonald, P.V., Layne, C.S., and Bloomberg, J.J. Transmission of locomotor heelstrike accelerations after space flight: Implications for head movement control. American Institute of Aeronautics and Astronautics, 1996 Life Sciences and Space Medicine Conference, Houston, TX, March 5-7, 1996.

Smith, S.L., Peters, B.T., Layne, C.S., McDonald, P.V., and Bloomberg, J.J. The effects of visual target distance on head movement control during locomotion. 20th Annual Meeting of the American Society of Biomechanics, Atlanta, GA, October 16-20, 1996.

Smith, S.L., Peters, B.T., Layne, C.S., Mulavara, A.P., Pruett, C.J., McDonald, P.V., and Bloomberg, J.J. An integrated approach to the measurement of locomotion strategies in astronauts following space flight. Annual Houston Conference on Biomedical Engineering Research. February, 1996.

Physiological Response During Descent on the Space Shuttle

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Funding:

Project Identification: 3.3.1
Initial Funding Date:
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration:
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

Task Description:

Additional information was not available in time for publication.

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 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

Task Description:

Additional information was not available in time for publication.

Morphological, Histochemical, and Ultrastructural Characteristics of Skeletal Muscle

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Funding:

Project Identification: 4.1.2

Initial Funding Date: 1993

FY 1996 Funding: $77,000

Solicitation: SMSP Phase 1A

Expiration:

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

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 bedrest 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. A comparison of muscle damage markers in the serum (CK activity and myoglobin) between sample obtained before and after flight showed no significant differences in these measured parameters.

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: 1996
Expiration: 1997
FY 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

Impaired thermoregulation, which has been observed during exercise following bedrest, may have significant impact during space flight operations by decreasing exercise capacity and orthostatic tolerance. Impaired temperature regulation would be manifested as higher levels of core temperature for a given oxygen consumption as a result of an attenuated cutaneous vasodilatory reflex and sweating response. Two male crew members of the Mir 18 mission performed supine submaximal cycle exercise (20 min 40% and 20 min 65% preflight VO_2pk), once preflight (145 days) and 5 days postflight. Postflight, neither crew member completed the exercise protocol, stopping at 28-29 min of exercise. The core temperature (Ingestible Telemetry Pill) at test termination was similar (37.8°C) for both subjects pre- and post-flight despite the shorter test duration postflight. The slopes of the skin blood flow (laser Doppler)/core temperature relationship (Subj 1: 396 vs 214; /Subj 2: 704 vs 143 Perfusion Unit/°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 $O(V,\bullet)O_2$peak, followed immediately by 20 minutes of exercise at 65% preflight $O(V,\bullet)O_2$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 was to be sampled every 30 seconds and stored in the HTI receiver. Heat production was to be calculated from $O(V,\bullet)O_2$ 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 (Perimed 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 member's 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 (subject 1) 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 crewmembers after short-duration shuttle flights. The addition of these crewmembers 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 in ground-based conditions in which heat loss responses are impaired (patients with inability to vasodilate appropriately such as those with hypertension, patients with impaired sweat responses, or workers or soldiers who wear impermeable clothing.)

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

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

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

FY96 Publications, Presentations, and Other Accomplishments:

Lee, S.M.C., Williams, W.J., Siconolfi, S.F., Gonzalez, R., Greenleaf, J.E., Mikhaylov, V., Kobzev, Y., and Fortney, S.M. Temperature regulation in crewmembers after a 115-day space flight. Experimental Biology '96, 1996.

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

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Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $

Solicitation: 94 OLMSA-01

Expiration: 9/96

Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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). 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.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Harris, B.A., Billica, R.D., Bishop, S.L., Blackwell, T., Layne, C.S., Harm, D.L., Sandoz, G.R., and Rosenow, E.C. The use of physical diagnosis for the practice of space medicine. Mayo Proceedings, (in press).

Trace Chemical Contamination of Spacecraft Air

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Funding:

Project Identification: 5.3
Initial Funding Date: 10/95
FY 1996 Funding: $135,130

Solicitation: US/RSA Negotiations
Expiration: 9/96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NASA 3 Onward
Responsible NASA Center: JSC
Flight Hardware Required: GSC, SSAS, FMK

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 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.

The atmosphere of Mir was evaluated for trace chemical pollutants during expeditions Mir 21 and NASA-2. In general, the atmospheric contaminants were greater than those found in the space shuttle, but the Mir contaminants levels still meet U.S. acceptability standards except for mucosal irritants. In particular, formaldehyde, a mucosal irritant and carcinogen was consistently found in concentrations 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 off-gassing and payload leaks. Data from NASA-2 indicate that spatial variations in pollution levels are generally less than 25%. Temporal variations are generally limited unless a

new source of air pollution, such as a new module opening or experiment startup, occurs. Results from canister samplers and the solid sorbent sampler are consistent; however, the Russian AK-1 sorbent system seems to yield high results. 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.

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.

Dynamics of Calcium Metabolism and Bone Tissue

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Funding:

Project Identification: 2.1.2
Initial Funding Date: 10/95
FY 1996 Funding: $240,000

Solicitation: US/RSA Negotiations
Expiration: 9/96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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.

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
Initial Funding Date: 10/95
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration: 9/96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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 n April in New Orleans and at the 12th Man in Space Symposium, Washington, DC.

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; the methods will also assist in studies of the physiological effects of physical activity.

Red Blood Cell Mass and Survival

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Funding:

Project Identification: 2.2.5
Initial Funding Date: 10/95
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration: 9/96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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.

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; the methods will also assist in studies of the effects of altitude on red blood cell metabolism.

Anticipatory Postural Activity During Long-Duration Space Flight

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P. Vernon McDonald, Ph.D.; KRUG Life Sciences, Houston, TX
Andrei A. Voronov, Ph.D.; All-Russian Scientific Research Inst. of Physical Culture

Funding:

Project Identification: 4.2.4
Solicitation: US/RSA Negotiations
Initial Funding Date:
Expiration:
FY 1996 Funding: $75,000
Students Funded Under Research: 1

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir), Mir-23 (as part of RSA)

Responsible NASA Center: JSC

Task Description:

The proposed project is designed to investigate the fundamental contributions of cutaneous and proprioceptive information in maintaining in-flight neuromuscular activation and postflight postural equilibrium. Developing appropriate in-flight countermeasures to maintain neuromuscular activation and minimize muscle atrophy will reduce the postflight postural control problems experienced by many crew members. The primary objective is to determine whether in-flight foot sensory input can be used to maintain 1-G neuromuscular activation patterns associated with arm movement. The secondary purpose is to determine the effect of long-duration space flight on postflight postural control responses and postural stability during arm movement.

The experimental protocol involves the crew members raising their arms as rapidly as possible before, during, and after flight. The inflight testing consists of four arm-raising conditions that are designed to vary the degree of foot sensory input. Arm movements are completed while the subject freefloats, freefloats with the addition of foot pressure, is secured passively at the feet to the Mir or Shuttle's support surface with Velcro™, and while connected via bungee cords to the support surface. Electrical activity (EMG) from selected arm, trunk, and leg muscles and arm acceleration is monitored. Muscle-activation latencies referenced to arm movement initiation are obtained, and temporal muscle activation patterns developed for each experimental condition. In this way, any changes in the neuromuscular activation characteristics associated with the experimental conditions are detected. During preflight and postflight testing, subjects perform the arm raises while standing on a force plate in order to obtain ground reaction forces and center of pressure (COP) measures. Body segment kinematic measures are also obtained. These measures enable determination of the degree of postflight postural instability associated with voluntary arm movement. As hypothesized, lower limb neuromuscular activity normally preceding arm movement during 1-G movements is eliminated while subjects freefloat but is restored when foot sensory input is available. It has also been shown that postural instability as measured by excursions of the COP increases relative to preflight values. For an in-flight experiment the project has proceeded smoothly, flying on STS-63, STS-71 (ground-based portion of protocol), Mir-18, Mir-19, and Mir-21. It is currently

flying aboard Mir-23 as part of the Russian Science Program. Data analysis is proceeding steadily and several preliminary reports have been made. The results are corresponding to the hypotheses.

What has been accomplished thus far? The project has flown aboard STS-63, STS-71, Mir 18, Mir 19, and Mir 21. It is currently flying aboard Mir 23 as part of the Russian Science Program. To date, nine subjects have completed the inflight portion of the experiment and eleven subjects have completed the ground-based experimental conditions. Of these subjects, eight have flown long-duration missions.

Data analysis of the inflight data has included the following:
A) Average arm accelerations have been normalized to peak accelerations obtained in the freefloating arm raise condition, and comparisons between the various inflight conditions have been made.
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 were 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 were 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.

What questions have been answered? 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 7-segment dynamical computational model of a human has been completed and has been used to perform "desktop" experiments. The results of these experiments will be presented at the International Society of Biomechanics in Sport Symposium (ISBS), Denton, Texas, June, 1997. This model uses algorithms which enable it to predict optimal solutions to a variety of movement tasks. A manuscript providing a detailed account of the inflight portion of the experiment is currenty in revision.

How does this year's progress affect future work on this task? 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 movement solutions for a particular task. Since the model allows for the changing of initial conditions (e.g. 20% loss of ankle muscular strength, limb amputation, restricted range of joint motion), it will be used to predict optimal movement outcomes for a variety of patient populations. Therapists can then design rehabilitation programs designed to reach the optimal functional state that can be achieved by a particular patient.

This project has the potential to increase our understanding of the processes whereby sensory input results in neuromuscular activation. It is suggested that many of the processes that contribute to muscle atrophy on Earth (i.e., muscle disuse, lack of sensory input) also contribute to the atrophy associated with space flight. It is anticipated that foot pressure will be regularly used to attenuate lower limb muscle atrophy and maintain the functional state of proprioceptive reflex loops in bedridden patients. Dynamic computational models will eventually be used to visualize and predict movement outcomes for both patient and athletic populations.

In addition to the benefits listed above, the dynamic computational modeling and devices which provide controlled patterns of sensory input will be integrated into virtual reality environments. Adding sensory input to the virtual environment will dramatically improve the fidelity of these environments for use as training tools. Computational models will eventually be introduced into the virtual environments to "discover" optimal solutions to a variety of tasks. Information gained from these predicted optimal outcomes will be incorporated into training protocols.

FY96 Publications, Presentations, and Other Accomplishments:

Layne, C.S., Bloomberg, J.J., McDonald, P.V., Mulavara, A.P., and Pruett, C.J. The use of foot pressure to enhance neuromuscular activation during space flight. Annual Meeting of the American Institute of Aeronautics and Astronautics, Houston, TX, March, 1996.

Layne, C.S., McDonald, P.V., Mulavara, A.P., Kozlovskaya, I.B., and Bloomberg, J.J. Adapting neuromuscular synergies in microgravity. Bernstein's Traditions in Motor Control Conference, Pennsylvania State University, University Park, PA, August, 1996.

Layne, C.S., McDonald, P.V., Pruett, C.J., Mulavara, A., Kozlovskaya, I.B., Voronov, A.V., and Bloomberg, J.J. The impact of space flight on anticipatory muscle activation. Annual Meeting of the American Institute of Aeronautics and Astronautics, Houston, TX, March, 1996.

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Mulavara, A.P., McDonald, P.V., Layne, C.S., Poliner, J., Pruett, C.J., and Bloomberg, J.J. Quantifying adaptive preparatory postural adjustments that occur following space flight. 14th Annual Houston Conference on Biomedical Engineering Research, Houston, TX, February, 1996.

Alterations in Postural Equilibrium Control Associated with Long Duration Space Flight

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Funding:

Project Identification:
Solicitation: US/RSA Negotiations
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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 5 crewmembers from joint US/Russian missions participated in pre- and postflight data collection sessions: 2 from Mir-21, and 3 from Mir-22/NASA-3. Preflight data were also obtained from 3 crew members currently aboard Mir-23/NASA-4.

Data Processing and Analysis

- Preliminary processing of the Paradigm 2 neuro-sensory control test data has been completed using off-the-shelf software. Comparisons of these long duration data with our existing short duration database are underway.
- 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 for both Paradigm 1 neuro-motor control test data and Paradigm 2 neuro-sensory control test data is complete for the Mir-21 crew, and in progress for the other data collected this year.
- A technique for estimating tonic ankle stiffness that was developed in-house to analyze data from another spaceflight experiment is currently being modified to be applicable to this data set. The results of this analysis should provide insight into the relative contributions of strength deficits and changes in neuro-sensory controls to postural ataxia.
- A custom software system to analyze the EMG data has been developed in Russia. Processing of one subject's pre- and postflight data is complete. Additional EMG analysis techniques to detect strategic changes in muscular coordination are under development in-house, and will be applied following fine tuning of the algorithms.
- 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 neuro-motor control EMG data.

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 crewmembers were that severely affected.
- The nature of postflight balance disturbances appears to be different following long duration than short duration space flight missions: in addition to problems of sensory integration and the inability to adequately use vestibular system inputs, long duration crewmembers appear also to be affected by lower level structural changes.
- 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, perhaps due the mechanistic changes hypothesized above.
- Now that the size of the long duration subject pool is more substantial, work is underway to categorize the differences between postural ataxia induced by long and short duration space flight, and to publish these data.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Paloski, W.H. Vestibulo-spinal adaptation to microgrvity. J. Otolaryn. - Head and Neck Surgery, (in press).

Paloski, W.H. Neural-biomechanical interactions affect postural control after space flight. Engineering Foundation Conference: Biomechanics and Neural Control of Movement, Mt. Sterling, OH, June 1-6, 1996.

Paloski, W.H. Vestibulo-spinal adaptation to microgravity. Invited Lecture at the Vestibular Dysfunction: Legacies and Lessions from Space Symposium sponsored by the American Academy of Otolaryngology-Head and Neck Surgeons with NASA and the National Institutes of Health. Washington, D.C., September 28, 1996.

Microbiology

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Funding:

Project Identification: 5.1
Initial Funding Date: 10/94
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration: 12/97
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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 aerobic microbiota of the two crew members were characteristic of healthy individuals. No remarkable change in the body-bioburden was apparent upon return to Earth except for the present of *A. niger* on the skin and throat swab of crew member 2. This fungus is commonly present in the terrestrial environment, and was also isolated, in rather large numbers, from Mir surfaces and air samples. It is obvious, therefore, that the fungus was a 'carry over' from Mir, and no undesirable health effects were noticed. One crew member was

clearly a carrier of *S. aureus* and completion of DNA fingerprinting will verify the suspected colonization of the other crew member.

The surface and water-borne bacterial and fungal loads were mostly within the limits set for the International Space Station. However, the fungal counts in the air tended to be higher. In general, the microbiological profile of the Mir was comparable to that of the Orbiter and Spacelab.

Accumulating evidence suggests that the human immune response may be attenuated during space flight. To control the development, transmission, and treatment of infectious diseases, the effects of space flight both on microorganisms themselves and on the human immune response must be understood. This study will help in adding to the body of knowledge with regard to the mode of action of microbial infection - a problem that is directly associated with immune compromised individuals on Earth.

Microbes' colonization of inanimate surfaces and hardware of the spacecraft can also lead to biodeterioration of critical life support instrumentation and equipment as well as the release of toxic volatiles. All these are problems associated with an Earth problem commonly called "sick building syndrome" (SBS) or "building-related illnesses." Reducing risk to SBS requires monitoring both the habitation environment and the occupants, such that the levels and types of microbes do not reach critical levels. A thorough understanding of the microbial population dynamics on board spacecraft will allow for development of predictive measures that can be used on Earth. The information gained from this study will be helpful in the design of future spacecraft as well as environmentally conscience buildings, and development of monitoring requirements in order to minimize microbial cross-contamination.

Viral Reactivation

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Project Identification: 2.4.3
Initial Funding Date:
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration:
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

Task Description:

Several strains of herpes virus are commonly found in humans. These viruses cause cold sores and other infections. Once a person is infected with the virus, it may be present for life and can be reactivated by several factors, including stress. Scientists believe that the stresses associated with space flight may increase the incidence of reactivation of latent herpes virus in crew members during a long-duration mission.

This study investigates the influence of space flight upon the frequency and magnitude of reactivation and shedding of clinically important latent viruses in saliva. Saliva samples were collected from the three Mir 18 prime and three backup crew members during a two month preflight period to establish baseline values. The objective of this experimen 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.

Research accomplished in FY96 included:

1) PCR methodology was capable of detecting latent viral DNA in saliva specimens collected and stored on the ground and in the Mir.

2) Neither crew member appeared to be shedding HSV 1.

3) Both crew members shed EBV before, during, and after the mission.

4) Shedding patterns of EBV of the two crew members were highly different during the inflight phase.

5) Even though the technical feasibility of the approach was clearly demonstrated, the low number of subjects prevent any major conclusions related to host-parasite relationships during space flight.

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.

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 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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.

An increase by at least 100% in the GITT (determined from breath hydrogen) during flight was observed in both crew members, indicating a decrease in GI motility. Both crew members, who repeated the test three times in-flight, exhibited a sustained increase in GITT throughout the duration of the mission. This is in agreement with an earlier observation of a 63% increase in the GITT during antiorthostatic bedrest.

Breath methane and hydrogen levels were several-fold higher during flight than on the ground. The source of methane, although unknown at this time, may be the crew. High breath methane and hydrogen levels are also associated with bacterial overgrowth in the GI tract and a possible proliferation of pathogenic bacteria, *Helicobacter pylori*. It was reported that the crew members received prophylactic treatment with bifidobacterium for disbacteriosis 60 days prior to flight. Their fecal bacteria flora immediately before flight were normal,

indicating that the disbacteriosis was effectively treated with bifidobacterium. No anomalies in the bacterial flora were noticed after return from flight (personal communication by Dr. Lizco, IBMP, Moscow). These results indicate that future studies should focus on examining some of these variables of the GI physiology during Mir station flights.

Hepatic function is key for the elimination of medications and toxicants from the body. It is governed by metabolizing enzyme activity and hepatic blood flow. The assessment of oxidative hepatic metabolism (hepatic function) was examined by measuring the antipyrine clearance in two Mir 18 crew members. Changes in hepatic function as a function of the duration of mission were assessed by comparing pre- and in-flight results. Data analysis indicates that hepatic metabolism was variable during flight. A more than 50% decrease in antipyrine clearance in one crew member and a 30% increase in the other were observed. Postflight, the clearance of antipyrine decreased approximately 20% in both crew members. A detailed pharmacokinetic analysis of the data is in progress.

The results of this experiment validate a non-invasive technique for the monitoring of GI motility and function. This technique will be useful for the clinical monitoring of geriatric and pediatric patients and for home health care organizations.

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 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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 (EDOMP), 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; and (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.

Gaze Phase 1A

Task Progress

Data Collection
•Mir-18 pre- and postflight data were collected on three subjects. Three preflight (120, 45, and 15 days before launch) and six postflight (0, 2, 6, 9, 12, and 64 days after landing) data collections were performed on two crewmembers. Three preflight (120, 45, and 15 days before launch) and five postflight (0, 2, 6, 9, and 64 after launch) were performed on one crewmember.
•Mir-19 pre- and postflight data were collected on two subjects. Three preflight (120, 60, and 15 days before launch) and six postflight (1, 5, 9, 64 days after launch) were performed.
•All Mir-18 and Mir-19 data were lost inflight The Gaze experiment was never performed because of a MIPS-3C failure.

Data Analysis Summary
Data analysis continues.

Preliminary Science Findings

Mir-18:
Due to time constraints and protocol reductions, responses can only be compared across all crew members for Predictable Target Acquisition (PTA) and Smooth Pursuit (SP) tracking at the 0.33 Hz frequency. Additional postflight responses on two crew members were obtained for Head Shakes and SP at additional frequencies. For the STS-71 crew member both target acquisition and smooth pursuit tracking were compromised. Recovery appeared rapid. Responses and recovery are directly in keeping with subjects participating in comparable measurements obtained as a part of the EDOMP program.

For the Mir crew members, both target acquisition and smooth pursuit were ***severely*** compromised. Postflight responses resembled those obtained late inflight for EDOMP crewmembers. Specifically, fine motor control of head movements was absent during rapid movement of the head relative to the trunk (even with feed-back of the heads position in space). In one crew member, gaze in response to targets outside of the effective oculomotor range (EOM) was possible only with direct neck input to the eye burst neurons. In the remaining two crew members gaze beyond the EOM (and often within the EOM) was established only with many saccadic eye movements, suggesting large changes in VOR gain; responses to both target acquisition and smooth pursuit in these two crew members resemble those of cerebellar patients (but are probably due to brainstem signal integration problems).

Only one Mir crewmember appeared to show a recovery to preflight responses parameters by R+9. Recovery for the additional two Mir crew was clearly not complete by R+9 (i.e., observation of online data appeared to show compromises in SP (multiple saccades) and the VOR). Even by R+12 these crew members still showed some response degradation.

Mir-19:
TBD

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 ensure the safety, health, and well being of future astronauts.

Greenhouse

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Funding:

Project Identification: 7.1.2
Initial Funding Date: 10/93
FY 1996 Funding: $974,107

Solicitation: AO-OSSA-84
Expiration: 9/95
Students Funded Under Research: 6

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: ARC

Task Description:

The Mir Space Station provides an outstanding opportunity to study long-term plant responses to microgravity. Furthermore, if plants can be grown to maturity in microgravity, they might be used in future life-support systems. The primary objective of the Greenhouse experiment was to grow a Super-Dwarf wheat through a complete life cycle in microgravity; i.e., from seed to seed. Additional objectives were to study chemical, biochemical, and structural changes in plant tissues as well as photosynthesis, respiration, and transpiration (evaporation of water from plants). Another major objective was to evaluate the suitability of the facilities on Mir for advanced research with plants. The Greenhouse experiment was conducted in the Russian/Bulgarian-developed plant growth chamber, the Svet, to which the U.S. has added an instrumentation system to monitor changes in CO_2 and water vapor caused by the plants (with four infra-red gas analyzers monitoring air entering and leaving two small plastic chambers). In addition, the U.S. instrumentation also monitors O_2; air, leaf (IR), and substrate temperatures; cabin pressure; photon flux; and substrate moisture (18 probes in the root module). Facility modifications were first performed during the summer of 1995 during Mir 19, which began after STS-72 left Mir. Plant development was monitored by daily observations and some photographs. Plant samples were collected five times during the 1995 experiment for chemical fixation or drying, and at final harvest. Samples were returned on STS-74 in November 1995. Because four of six light sets failed at the beginning of the experiment, plants grew very poorly; no seed heads were formed. The experiment was repeated in 1996 as part of NASA 3, using a new lamp bank and other equipment. Samples were returned on STS-81 in January 1997. The plants grew extremely well, producing far more biomass than in any other plant experiment in space, but seeds failed to form in the ca. 280 heads that developed. At present, it

appears that failure of seed formation was caused by ethylene, a gaseous plant hormone, in the Mir cabin atmosphere.

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

During this reporting period, we carried out ground studies to see if we could produce vegetative plants like those from the Mir 19 experiment. So far, our plants (in Utah) produce heads even if the light levels are so low that the plants die. A detailed simulation experiment is being carried out by David Bubenheim at ARC, duplicating most environmental parameters of the Mir 19 experiment. Again, plants have formed heads although they are close to death as this is being written. In the 1996 experiment (NASA-3), seeds were planted in Svet on Mir on August 5 with four samplings for chemical fixation or drying and final harvest on December 6. Samples and hard discs were returned on STS-81 on January 22, 1997. The experiment was highly successful in that equipment functioned almost flawlessly and Super-Dwarf wheat plants grew vigorously, producing more biomass than has ever been produced by plants in space. The plants were only moderately disoriented, apparently growing mostly toward the lights. Because there was unusually extensive tillering (branching at the base), there were many stems, and virtually all of these produced heads, a total of about 290. Unfortunately, not a single seed was formed in all of those heads. At present, a strong case can be made that this failure to set seeds was a response to ethylene gas in the cabin atmosphere. Ethylene, which is harmless to humans until it reaches asphyxiation levels, is a gaseous plant hormone that influences many plant responses at concentrations in the parts-per-billion range. The gas is known to cause sterility in cereals including wheat, and it also causes extensive tillering. Measurement of ethylene in gas samples returned from Mir show concentrations well above those thought to affect seed formation in wheat. We are currently setting up experiments to test the ethylene hypothesis. It is clearly premature to conclude that sterility in wheat can be caused by microgravity, but our results raise a red flag about ethylene for all future plant experiments in closed environments.

Fixed, dried, and frozen samples are being examined at Utah State University, ARC, and at the Institute of Biomedical Problems in Moscow.

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

FY96 Publications, Presentations, and Other Accomplishments:

Bingham, G.E., Brown, S.B., Salisbury, F.B., Campbell, W.F., Carman, J.G., Jahns, G., Bubenheim, D.B., Pletcher, D., Yendler, B., Sytchev, V., Levinskikh, M.A., Podolski, I., Ivanova, I., Kostov, P., and Sapunova, S. (abstract) Environmental measurements observed during the Greenhouse-2 experiment on the Mir Orbital Station. 31st Scientific Assembly of COSPAR, 14-21 July, 1996, p. 364.

Campbell, W.F., Strickland, T., Bubenheim, D., Salisbury, F.B., Hole, P.S., Gillespie, L., Levinskikh, M., and Ivanova, I. (abstract) Comparison of long-term storage in chemical fixatives on morphology and anatomy of Super-Dwarf wheat. 31st Scientific Assembly of COSPAR, 14-21 July, 1996. p. 365.

Gillespie, L.S., Salisbury, F.B., Campbell, W.F., and Hole, P. (abstract) Why were Super-Dwarf wheat plants grown in Space Station Mir vegetative: Heat shock, short day, or microgravity? ASGSB Bull., 10 (1), 74.

Levinskikh, M.A., Ivanova, I.E., Neiedova, E.L., Sytchev, V.N., Ilyina, G.M., Timonin, A.K., Vorobyevy, G., Salisbury, F.B., Bingham, G.E., and Brown, S.B. (abstract) Peculiarities of Super-Dwarf wheat growth and development in Greenhouse Svet in ground and space experiments. 31st Scientific Assembly of COSPAR, 14-21 July, 1996, p. 364.

Salisbury, F.B. "Life sciences: Botany" in "Yearbook of Science and the Future 1997." Encyclopaedia Britannica, Inc., pp 333-338, 1996.

Salisbury, F.B. "Life sciences: Botany" in "Yearbook of Science and the Future 1998." Encyclopedia Britannica, Inc., (in press).

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Salisbury, F.B., Bingham, G.E., Campbell, W.F., Carman, J.G., Hole, P., Gillespie, L.S., Sytchev, V.N., Podolsky, I.G., Levinskikh, M., Bubenheim,D.L., and Yendler, B. (abstract) Experiments with Super-Dwarf in Space Station Mir. ASGSB Bull., 10 (1), 34.

Salisbury, F.B., Campbell, W.F., Carman, J., Bingham, G., Bubenheim, D.L., Yendler, B., Sytchev, V., Levinskikh, M.A., Ivanova, I., Chernova, L., and Podolsky, I. (abstract) Plant growth during the Greenhouse II experiment on the Mir orbital station. 31st Scientific Assembly of COSPAR, 14-21 July, 1996, p. 364.

Salisbury, F.B., editor. "Units, Symbols, and Terminology for Plant Physiology." Oxford University Press/New York, Oxford, pp 234, 1996.

Sytchev, V.N., Gurieva, T.S., Levinskikh, M.A., Podolski, I.G., Souza, K.A., Jahns, G.C., Pletcher, D.L., Salisbury, F.B., and Bingham, G.E. (abstract) Fundamental biology investigations (FBI) of the "Mir-Shuttle" project (SLM-1): Plans and implementation. 31st Scientific Assembly of COSPAR, 14-21 July, 1996, p. 364.

Humoral Immunity

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Funding:

Project Identification: 2.4.2
Initial Funding Date: 10/ 95
FY 1996 Funding: $42,000

Solicitation: US/RSA Negotiations
Expiration: 9/ 96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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 isotopes (type 3, type 7F, type 9N, and type 14). The samples were also analyzed for the levels of serum immunoglobulins (IgG, IgA, IgD, IgE, and IgM). Analysis of salivary IgA levels (antigen specific and total) and lysozyme levels will be performed in batch with the Phase 1B samples.

While the data are limited, some trends are apparent. The three crew members all exhibited minor shifts in the levels of isotope specific antibodies between the preflight baseline and the inflight baseline. In general, these do not appear to be highly significant, and they suggest the serum antibody levels are not altered directly by space

flight. The immunized crew members did respond to the vaccine by increasing antigen-specific antibody titers. However the degree of response may be altered for some isotopes in some individuals. General immunoglobulin levels did not change significantly during or after flight in any of the crew members. This was consistent with previous observations.

The data from the Mir-18/STS-71 constitute the first part of this investigation. The data were intended to include additional subjects on Mir-19. However, the Mir-19 crew did not participate in this experiment. 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
Initial Funding Date: 10/95
FY 1996 Funding: $45,000

Solicitation: US/RSA Negotiations
Expiration: 9/ 96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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.

The circulating subpopulations of peripheral white cells were not greatly altered during flight as compared to the preflight baselines. In contrast, the samples taken within 2-3 hours after landing had the relative granulocytosis and lymphopenia observed after most space flights. A relative decrease in T cells, monocytes, B cells and NK

cells was also noted after flight. These changes were not observed in the inflight sample taken during the last flight day, suggesting that they are an acute response to reentry and readaptation to unit gravity.

In vitro activation of lymphocytes isolated from crew members during flight or immediately after flight was not significantly different from the control periods as determined by the expression of early activation markers. Expression of CD 69 protein or IL-2 receptor (CD 25) 24 hours after mitogen activation was not altered relative to preflight control samples. All crew members exhibited good NK cell function on landing day; however, variable decreases in cytotoxicity were noted 9 days after landing. The reasons for this effect are currently unclear, though it is consistent with previous observations during long duration space flight on Mir.

Statistical significance is limited by the low sample number of the study; however, some interesting trends are suggested by the current results. It appears that the major phenotypic changes which are observed after landing arise as a result of reentry and reambulation. Another interesting observation was that expression of early activation markers in cells activated *in vitro* immediately after flight was not substantially different from the preflight values. However, these data do not directly assess proliferation and inhibition further downstream in the activation sequence is possible. Further research will be required to determine the importance of these findings.

This task is focused on the examination of the effects of space flight on human immune cells. This investigation will, however, provide insight into mechanisms which regulate human immune function. These studies will improve the understanding of the effects of psychological and physical stress on specific components of the cellular immune system. The examination of specific cell functions and changes in the cytokine patterns should be particularly relevant to the regulation of immune responses in health and disease.

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 1996 Funding: $89,385
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

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 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 postflight response with preflight values to determine how long (and the mechanisms used) to readapt to Earth's gravity. The test 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.

The following tasks were undertaken in fiscal year 1996:

- 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
Initial Funding Date: 10/94
FY 1996 Funding: $95,938

Solicitation: US/RSA Negotiations
Expiration: 9/95
Students Funded Under Research: 0

Flight Information:

Flight Assignment: SLM-1A, (Mir-18/STS-71)
Responsible NASA Center: JSC

Task Description:

Several exercise techniques 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 yet been done, but 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 maintain 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 basis. 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 1996 Funding: $

Students Funded Under Research: 0

Joint Agency Participation: N/A

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: Urine Collection Device (UCD); Bar Code Reader (BCR)

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 completed for the Mir-18 mission. Inflight dietary data were missing on 2 out of the 3 crew members. Only one crew member had recorded inflight dietary intake on 2 of 3 potential diet monitoring dates. The results of the dietary data on the risk of renal stone formation cannot be determined due to the missing data.

Preliminary results have suggested the following conclusion: 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 crewmembers exposed to microgravity. The first phase of this investigation will assess the direct effects of microgravity on this potential during long duration space flight. Following this assessment, proven Earth-based therapies will be recommended to protect the health and well-being of the 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. This advanced technology is scheduled as a technology demonstration on the NASA-6/NASA-7 and Mir-25 missions.

Measurements of Cytogenetic Effects of Space Radiation

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Funding:

Project Identification: 5.2.6
Initial Funding Date: 10/94
FY 1996 Funding: $

Solicitation: US/RSA Negotiations
Expiration: 9/95
Students Funded Under Research: 2

Flight Information:

Flight Assignment: SLM-1A, (Spacelab-Mir)
Responsible NASA Center: JSC

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. 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 $2.8\pm0.99\times10^{-3}$, $8.1\pm1.33\times10^{-3}$, and $7.02\pm1.6\times10^{-3}$ 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 2.9, since the absorbed dose measured by physical dosimeters is 4.99 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 risks to workers accidentally exposed to radiation.

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: 10/94
Expiration: 4/96
FY 1996 Funding: $122,846
Students Funded Under Research: 0

Flight Information:

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

Responsible NASA Center: JSC

Flight Hardware Required: Holter Monitor, ABPM, Barocuff Device, Portapres

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-Is Assigned to this Task

Funding:

Project Identification: E120
Initial Funding Date: 1/96
FY 1996 Funding: $126,250

Solicitation: 78 AO
Expiration: 12/96
Students Funded Under Research: 3

Flight Information:

Responsible NASA Center: JSC

Task Description:

The tasks for the final year of this project were to complete the analytical work and write 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 principle findings were:

(i) 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.

(ii) 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.

(iii) energy intake is less during flight than preflight. Preflight, the mean energy intake was 39.0 ± 2.5 (10) kcal/kg/d. There was constant at 30.4 ± 1.5 (12) kcal/kg/d for the remainder of the flight period ($p < 0.05$.)

(iv) 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 on 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 on the 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 should be made, though, about long flights from this short-term data.

FY96 Publications, Presentations, and Other Accomplishments:

Stein, T.P., Leskiw, M.J., and Schluter, M.D. Diet and nitrogen metabolism during spaceflight on the Shuttle. J. Appl. Physiol., vol. 81, 82-97 (1996).

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 1996 Funding: $298,073

Students Funded Under Research: 11

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94) and NIH-R2 (STS-70, 6/95)

Responsible NASA Center: ARC

Task Description:

Dr. Alberts and colleagues will study the fetal and postnatal development of rats to verify the hypothesis that microgravity reduces stimulation of the developing fetal vestibular system and thereby alters early function. The studies will also emphasize the behavior and physiology known to contribute to successful pregnancy, labor, delivery, and onset of postnatal maternal care, especially lactation.

The data expected can contribute to our understanding of basic vestibular function. This function plays an important role in numerous disorders of movement and coordination, rehabilitation processes after injury, and deterioration during aging. The data expected will also answer fundamental questions regarding mammalian development and pregnancy in space and the effects of weightlessness on birth and lactation.

Our progress thus far has been excellent; the experiments have yielded new and exciting observations. The maternal studies produced the first continous records of rat behavior during initial readaptation (R+60 hrs) to 1-G. Offspring were analyzed prenatally and postnatally. Prenatal status of vestibular function was assessed in fetuses immediately following space flight and during the early postnatal period, which began about 2 days after recovery. We used a special preparation for fetal externalization and assessed prenatal vestibular responsivity by measruing autonomic (cardiac) reactions to angular acceleration (tilt). Postnatal testing emphasized vestibular function and its development. Tests included surface righting, non-contact righting in a water drop test, and head position responses to rotation. Together, this program provided a broad yet detailed view of the mother offspring system after nine days of space flight during the last half of the rats' 22-day pregnancy.

The R2 findings revealed many of the kinds of results that researchers in developmental biology have long anticipated. Some were as predicted. Other findings were contrary to predictions, and thus may be even more exciting.

Inflight behavior of pregnant rats. We analyzed the behavior of Flight dams from the in-flight videorecords and of the Synchronous control dams housed under flight-like conditions. From these videorecords, we could

describe individual and social behavior, quantify behavior in a variety of ways, and analyze kinematics of individually marked animals in the AEMs. We observed feeding, drinking, and social and self-grooming in the group-housed pregnant rats in space and on Earth. Activity levels, by certain measures, were equivalent. Body weight gain during the flight was excellent for the dams. This was an important finding in light of the severely compromised body weights of pregnant rats flown previously on Cosmos 1514.

Post-flight status of pregnant dams. One-minute samples of early post-recovery behavior were used to characterize the pregnant dams' early responses and readjustments to 1-G. Our analyses confirmed the familiar profile of a hypoactive animal that is posturally depressed. Flight dams were posturally depressed: their quadrapedal stance was lower, and their heads were not lifted as much as or for as long as were the Synchronous controls'. Even the Flight dams' tails were less elevated from the substrate. Overall, Flight dams reared less frequently, and when they did, rearing was brief and tended to involve less extension of the soleus. Flight animals more often used a wall for support or balance during rearing. They also ambulated less.

Readaptation to 1-G. Immediately after the post-flight video, rat dams were housed individually in observation chambers where they were kept under continous video surveillance. The timelapse and real-time recordings were the basis of analyses of the timecourse of behavioral readaptation. Flight animals continued to exhibit relative lethargy, but their feeding and drinking behavior were at normal levels. Ambulating, rearing, nest-building, and self-grooming were decreased. There was a measurable circadian rhythmicity to their behavior, however, and this became increasingly pronounced until about 60 hr after Recovery, which was about the time of labor onset in the rats. The dams' labors obscured other activity measures, and we turned our attention to the quantification of labor and vaginal delivery.

Birth and lactation. As forecast by the stunning results of NIH-R1, we witnessed non-complicated vaginal births, on schedule, that yielded offspring equivalent in number and size to those of the controls. R2 was an important study, however, because the dams in the sample had not sustained an immediate post-flight abdominal surgery as in R1. Thus, the R2 data stand as the definitive evidence of the patency of rat parturition after 9 days of space flight deconditioning.

We quantified the number of labor contractions displayed by dams in the 6 hr prior to delivery. Flight dams exhibited significantly more labor contractions than did Synchronous controls, a finding that agrees with observations made of the R1 dams as well. Thus, space flight appears to exert some of the predicted effects of weakened striated muscle, an interpretation supported by preliminary muscle histology, as described by R2 investigators Wassersug and Fejtek. There are pragmatic issues raised by these findings that pertain to anticipated use of long-term platforms such as the Space Station for mammalian reproduction. It is now demonstrably imperative that we study systematically the course of altered functionality of abdominal and uterine muscle to determine whether vaginal birth can be sustained with longer space adaptation.

Mammary gland morphologic and metabolic activity measurements made in Flight and Control dams indicated that space flight did not interfere with the ability of pregnant rats to produce milk to support their offspring. Elevated metabolic activity was observed in mammary tissue of Flight dams shortly after landing on Day 20, but not in postparturient (Day 22) dams.

Fetal vestibular function. R2 provided the opportunity for unique investigations of fetal vestibular function immediatley after space flight. Five Flight dams and an equivalent sample of Synchronous and Vivarium controls provided prenatal subjects for testing. Briefly, we applied a procedure wherby fetuses are gently externalized from the uterine born keeping umbilical and placental connections intact. The fetus is maintained in a warm, isotonic solution where it can be fitted with subcutaneous electrodes for EKG. It is then placed within a tilt apparatus. We have previously shown that cardiac decleration, classical psychophysiological measure of sensory detection, can be used reliably in fetal rats. This method was successfully applied in R2. Contrary to many predictions, we observed highly robust responses to the tilt stimulus (considered as a roll movement due to the fetus' orientation). In fact, the Flight fetus' responses were more dramatic than were the controls. One explanation is that the space flight conditions increased their responsivity. Another explanation is that the

Flight animals were displaying a developmentally different response than the controls. We are presently conducting tests to help select the approriate kind of explanation.

Postnatal vestibular function. We used a battery of tests similar to those used in R1 to evaluate vestibular function in postnatal offspring. As in R1, surface righting appeared normal. We also replicated the R1 finding that Flight pups in the water drop tests, which we believe involves the otoliths, displayed disrupted performance in early tests. Three days later, their deficits were not seen. Finally, the rotation test, a labyrinth challenge, revealed different response patterns in Flight vs. control pups that also were absent in older (5-day) offspring.

The postnatal results underscore our new view of vestibular "tuning," that is a dynamic process adaptation during which the vestibular system responsivity is continuously set and maintainedby tonic stimulation. Thus, the pups' postflight changes reflect a shit from inflight profiles of stimulation to those imposed by the 1-G, Earth-normal enviornment. This view does not exclude the possibility of a "critical period" in vestibular development. Our interpretation is presently limited by the window provided by the current flights. If different ages are studied in future experiments, we may see a change in the dynamics of the system.

Implications of in-flight dam behavior on vestibular function of offspring. The most dramatic difference found in the behavior of Flight dams relative to controls was the number of times the dams moved by rolling. This appeared to be caused by their natural tendency to ambulate across all surfaces and thus roll on their long axis. Thus, prenatal offspring reside under remarkable conditions. The prenatal otolith is effectively unloaded in conditions of orbital flight, whereas their labyrinths are seemingly *hyper*-simulated through the altered behavior of the dam. This observation has a compelling fit with our observation of dramatic HR responses to labyrithine stimulations in the fetuses, to NIH-R2 brainstem anatomy (Fritzch and Bruce) and to specific alternations in abdominal musculature described by NIH-R2 histology (Wassersug and Fejtek).

The research conducted as part of NIH-R1 was primarily and foremost a series of investigations into basic biological processes. Many of the observations verified and extended our NIH-R1 findings. In both investigations, the basic biological processes under consideration relate to (a) the ability of the body of an adult female mammal to tolerate space flight challenges and maintain normal gestation, followed by vaginal delivery during 1-G readaptation, and (b) the developmental status of a vestibular system that forms and begins to function in microgravity, i.e., in the absence of normal gravitational forces. Within each of these pursuits are embedded numerous more specific yet fundamental research issues.

Most basic biological studies bear on some practical considerations, and this is true of the R1 and R2 studies. In particular, these experiments provide a foundation for understanding how the vestibular and proprioceptive systems are established in mammals and how function is shaped and maintained throughout life. Naturally, the results of a single experiment only give a most preliminary glimpse, but the ramifications are immense. Vestibular function and dysfunction appear early in human life — beginning with births and then through the maintenance of posture and coordination. Fetuses with vestibular disorders are prone to breach birth. The elderly suffer many disastrous falls, many of which appear related to altered vestibular or proprioceptive function. Basic developmental studies utilizing gravitational manipulations give new insights into the forces that shape and maintain the vestibular system, and will undoubtedly contribute to the foundation of knowledge needed for effective treatments and therapies.

One practical aspect of this work applies to the utilization of the upcoming international space station. Most plans for the life sciences laboratories on the space station include reproductive and developmental studies. As we learn more about the female mammal's adaptive responses to space flight, we can better plan the facilities needed for developmental research on a long-duration facility such as the space station.

FY96 Publications, Presentations, and Other Accomplishments:

Alberts, J.R. and Ronca, A.E. Behavior of pregnant rats in space: A key to interpreting spaceflight effects on perinates. Dev. Pyschobio., (in press).

Alberts, J.R. and Ronca, A.E. Pregnancy and parurition in rats following spaceflight. American Psychological Society, Symposium on Mammalian Development During Spaceflight, San Francisco, CA. June 1996.

Alberts, J.R., Burden, H., Hawes, N., and Ronca, A.E. Sampling both prenatal and postnatal offspring from individual rat dams enhances animal utilization without compromising development. Contemportary Topics in Lab. An. Sci., 35 (6), 61-65 (1996).

Alberts, J.R., Ronca, A.E., Abel, R.A., and Farrell, W.J. Vestibular tests of postnatal rats gestated during spaceflight. Dev. Psychobio., 29(2), 278 (1996).

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S., and Galvani, C.D. NIH.R2 spaceflight effects on rat parturition and offspring vestibular function. ASGSB Bull., 10(1), 29 (1996).

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S., Farrell, W.J., and Galvani, C.D. Maternal behavior and offspring development of NIH.R1 rats. ASGSB Bull., 9(1), 96 (1996).

Alberts, J.R., Ronca, A.E., Abel, R.A., Armbruster, M.E., Cabell, K.S., Farrell, W.J., and Galvani, C.D. Video images of vestibular tests of postnatal rats gestated during spaceflight. ASGSB Bull., 9(1), 84 (1996).

Plaut, K., Alberts, J.R., Maple, R.L., Muniam, S., Darling, A.J., and Casey, T.M. Mammary metabolism is altered in pregnant rats subjected to spaceflight (NIH.R2). ASGSB Bull., 10(1), 32 (1996).

Ronca, A.E., Alberts, J.R., Abel, R.A., and Farrell, W.J. Pregnancy and parturition of rat dams onboard the Space Shuttle. Dev. Pyschobio., 29(73), 296 (1996).

Ronca, A.E., Alberts, J.R., Abel, R.A., Armbruster, M.E., Cabell, K.S., Farrell, W.J., and Galvani, C.D. Spaceflight effects on rodent pregnancy and parturition. ASGSB Bull., 9(1), 96 (1996).

Ronca, A.E. and Alberts, J.R. Vestibular function in perinatal rats gestated in microgravity. Dev. Pyschobio., (in press).

Phantom Torso

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-07

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 0

Flight Information:

Flight Assignment: STS-95 [target]

Responsible NASA Center: JSC

Task Description:

Additional information was not available in time for publication.

Effects of Space Flight on Neuromuscular Development

Principal Investigator:

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Congressional District: -

Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 106-50-06
Initial Funding Date: 10/94
FY 1996 Funding: $0
Joint Agency Participation: NIH

Solicitation: 93-OLMSA-03
Expiration: 6/96
Students Funded Under Research: 4

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)
Responsible NASA Center: ARC
Flight Hardware Required: AEM

Task Description:

Dr. Bodine 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.

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.

Information regarding specific progress during FY96 was not provided by the principal investigator.

Investigations of the Effects of Microgravity on In Vitro *Cartilage Calcification*

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Funding:

Project Identification:

Initial Funding Date: 2/94

FY 1996 Funding: $115,124

Joint Agency Participation: NIH

Solicitation: 93-OLMSA-04

Expiration: 1/96

Students Funded Under Research: 2

Flight Information:

Flight Assignment: NIH-C2 (STS-66, 11/94)

Responsible NASA Center: ARC

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.

The experiment was again repeated on the STS-77 flight. Even with problems with temperature controls (range 33-36°), the results of the c-2 experiment were confirmed.

The cells in the flight cultures did not mature, formed few nodules, and showed no evidence of mineral depositon up to a culture age of 28 days. Ground controls showed the presence of mineral (based on chemical, spectroscopic, and histochemical analysis) by 21 days.

This study focuses on how cells regulate biomineralization. Results should provide insight into an extremely prevalent disease, osteoporosis. Although much of osteoporosis is associated with alterations in hormonal levels, disuse osteoporosis is not uncommon. Astronauts lose bone mass during short-term flight, and this "osteopenia" may not be different from the osteopenia that leads to increased fractures (osteoporosis). The research is designed to allow for understanding of the underlying mechanism of biologic calcification. When this is known, improved therapeutics may be developed; however, the flight research does not test therapeutic modalities. The benefit to the citizens in the US should be a clearer understanding of why bone loss occurs, the importance of weight bearing for prevention of osteoporosis, and in the future, the development of therapies to prevent fractures in an ever-growing elderly population.

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 1996 Funding: $ 106,544
Students Funded Under Research: 4

Flight Information:

Flight Assignment: HP-01, STS-89
Responsible NASA Center: JSC

Task Description:

The payload proposes to determine the stability and accuracy of cognitive and psychomotor performance across work shifts, to measure the subjective responses of crew members on emotion and disposition questionnaires across work shifts, and to determine the relationship between subjective responses and cognitive and psychomotor performance.

Progress over the past year has focused on completion of the software development and a pretest of the performance program with three male and three female participants during 20- to 30-minute work intervals twice each day (AM & PM) over a three to four week interval. Significant differences between males and females were observed in selective components of the performance battery, and results were reported in a presentation at the Annual Scientific Meeting of the Pavlovian Society.

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 in the interest of developing a valid and reliable early warning system for timely intervention of countermeasures to performance decrements.

Starch Metabolism in Space-Grown Soybean Seedlings

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Co-Investigators:

James A. Guikema, Ph.D.; Kansas State University

Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $

Solicitation: 93 OLMSA-05

Expiration: 9/96

Students Funded Under Research: 3

Flight Information:

Flight Assignment: BRIC-03 (STS-63, 2/95)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

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, data analysis and publication of the results were the main activities (see publication list). In the future, we will continue to try to elucidate the mechanism for reduced starch concentration in space-grown plant tissue. These studies will focus on the regulation of the rate limiting enzyme ADP glucose pyrophosphorylase which was lower in the space-grown soybean cotyledons. We will investigate the structural properties of starch grains formed in space tissue (soybean cotyledons and potato tubers). Experiments will be conducted to understand the interaction on ethylene and gravity in controlling the partitioning of biomass and metabolism 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.

FY96 Publications, Presentations, and Other Accomplishments:

Brown, C.S., Tripathy, B.C., and Stutte, G.W. "Photosynthesis and carbohydrate metabolism" in "Plants in Space Biology," Suge, H., ed. Tohoku University Press, Sendai, Japan, 127-134 (1996).

Brown, C.S., Sanwo, M.M., Hilaire, E., Guikema, J.A., Stryjewski, E.C., and Piastuch, W.C. Starch metabolism and ethylene production in space-grown soybean seedlings. American Society for Gravitational and Space Biology annual meeting, Charlotte, NC, October 1996.

Brown, C.S., Tibbitts, T.W., Croxdale, J.G., and Wheeler, R.M. Potato tuber formation and metabolism in the spaceflight environment. SAE Paper #96-1393, (1996).

Croxdale, J.G., Cook, M.E., Tibbitts, T.W., Brown, C.S., and Wheeler, R.M. (abstract) Structural aspects of potato tubers formed in space. ASGSB Bull., 10, 13 (1996).

Hilaire, E.M., Peterson, B.V., Guikema, J.A., and Brown, C.S. Clinorotation affects soybean seedling morphology and ethylene production. Plant & Cell Phys., 37(7), 929-934 (1996).

Sanwo, M.M. and Brown, C.S. Starch metabolism in space-grown soybean cotyledons. Plant Physiology annual meeting, San Antonio, TX, July 1996.

Tibbitts, T.W., Croxdale, J.G., Brown, C.S., and Wheeler, R.M. Growing potato tubers in space. HortScience, 31(4), 607 (1996).

Tibbitts, T.W., Croxdale, J.G., Brown, C.S., and Wheeler, R.M. (abstract) Potato tuber growth and starch accumulation in space. ASGSB Bull., 10, 28 (1996).

The Interaction of Microgravity and Ethylene on Soybean Growth and Metabolism

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $

Joint Agency Participation: NSAU

Solicitation:

Expiration: 9/96

Students Funded Under Research:

Flight Information:

Flight Assignment: CUE (STS-87, 11/97)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

Task Description:

Space flight has profound effects on plants including altered starch metabolism, increased ethylene production, and a change in biomass partitioning. However, the mechanism for and/or relationship between these changes are not clear. Therefore, as part of the Collaborative Ukrainian Experiment (CUE) we will test the hypothesis that ethylene concentrations will be enhanced in the space-grown soybean seedlings. This will result in diminished root growth in these plants relative to the ground-controls. Removal of the ethylene from the atmosphere around the plants will result in biomass partitioning similar between spaceflight and ground control. Further, we hypothesize that starch concentrations in the cotyledons will be reduced in the space-grown plants as a result of diminished AGPase activity. The level at which this enzyme is regulated will be investigated. We also hypothesize that the removal of ethylene from the atmosphere of the space-grown plants will result in starch concentrations similar to the ground-controls.

During this fiscal year the CUE project was established and the experiments were selected and defined. Progress was made on the definition of the protocol and hardware configuration for this experiment. This includes the determination of an appropriate method of ethylene removal from the canisters, a gas sampling and storage protocol, a method for initiating the experiment on-orbit, and the establishment of the timeline for in-flight activities. A Science Verification Test was conducted and Payload Specialist training took place. In the future we will participate in a Payload Verification Test to establish the final protocols for the mission. We anticipate the experiment will take place on STS-87 in October 1997.

Results from these experiments, in addition to fostering a climate of international cooperation in space, will lead to a more complete understanding of the influence that spaceflight has on plant growth and metabolism. This is of critical importance as humans venture deeper (and longer) into space. Long-term missions will utilize plants as part of a life support system for the crew, and the influence that space flight has on plants must be understood. The information generated by these studies will not only result in a more thorough understanding of

the influence of adverse conditions on plant growth and metabolism but will also supply important information toward the development of a life support system using plants.

Physiological Anatomical Rodent Experiment (PARE) 04: Flight Support

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Funding:

Project Identification: 5-01161

Initial Funding Date: 4/94

FY 1996 Funding: $86,467

Joint Agency Participation: NIH

Solicitation: 93-OLMSA-03

Expiration: 4/96

Students Funded Under Research:

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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.

Additional work accomplished during 1996 includes the completion of the analysis of plasma concentrations of progesterone. This completes all analyses on the project. The manuscript describing the completed study is currently in press, scheduled for publication in the March 1997 issue of *Journal of Reproduction and Fertility.*

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.

FY96 Publications, Presentations, and Other Accomplishments:

Burden, H.W., Zary, J., Russ, S., and Lawrence, I.E. Effects of space flight during pregnancy on rat ovarian function. ASGSB Bull, 10(1), 74 (1996).

Poole, M.C., Russ, S., Glover, B., Zary J., and Burden, H.W. Effects of space flight during pregnancy on rat uterine morphology. ASGSB Bull, 10(1), 46 (1996).

Effects of Space Flight on Muscles and Nerves

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Funding:

Project Identification:

Solicitation: 93-OLMSA-03

Initial Funding Date: 5/94

Expiration: 4/96

FY 1996 Funding: $

Students Funded Under Research: 1

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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 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, bedrest 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.

Work is ongoing with these flight tissues from STS-66. New antibodies are being used to answer questions regarding the presence or absence of connective tissue in the spaces among muscles in the flight tissues. Our data indicate that there is no connective tissue formation in the space between muscles that seems to appear during formation in microgravity conditions. In addition, sections are being stacked to reveal three-dimensional aspects of the newly formed muscle and spaces within.

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. α-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. $MLC_{1/3\ fast}$ 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 the reverse of the hypothesis.
   c. MHC_{peri} 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.

Work is continuing on these sections using additional RNA probes from earlier time points in development as well as antibodies against the resulting proteins in some case in order to understand changes in protein translation as well as transcription following development in microgravity.

Additional experiments are onging in the laboratory using *in vitro* muscle growth systems to begin to study the direct effects of development in microgravity on muscle by removing possible complications of the effects of microgravity on the adult animal during gestation. These experiments, in addition to the use of the Centrifuge facility at Ames Research Center, will broaden the possibilities for interpretation of the original flight data.

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.

Development of Sensory Receptors in Skeletal Muscle

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 5/96

FY 1996 Funding: $87,542

Joint Agency Participation: NIH

Solicitation: 93-OLMSA-03

Expiration: 4/97

Students Funded Under Research: 7

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

Task Description:

In this study of rats that underwent part of their prenatal development in space, we are examinining microscopically the formation of encapsulated sensory receptors — the two major types being muscle spindles and tendon organs — in hindlimb skeletal muscles. We are determining the presence, number, and size of the muscle spindles when the rats are of different ages (i.e., fetal or gestational day 17, new born, and adult or postnatal day 100). This research also has tested for 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 produce. Comparisons are made with similar measures on ground-based control rats.

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.

In the first several hours after return to Earth gravity, pregnant rats were more inactive than pregnant, Earth-bound control 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 present observations show that muscle spindles and tendon organs do develop by adulthood in hind limb extensor and flexor muscles of rats exposed to near-zero gravity during much of the latter half of gestation.

For rats that were at postnatal day 100, we found no significant differences from controls for muscle spindles within the soleus, an ankle extensor muscle, in terms of: 1) average number per muscle; 2) distribution along

the length of the muscle; 3) cross-sectional size of the spindle; 4) number and size of intrafusal muscle fibers; and 5) presence of sensory and motor nerve endings on intrafusal fibers. Muscle spindles have also been identified in the newborn flight (and control) rat's extensor digitorum longus muscle.

Studies are presently ongoing to characterize further the muscle spindles within the soleus and extensor digitorum longus muscles of newborn flight and control rats as well as the extensor digitorum longus muscle of adults.

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. 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.

FY96 Publications, Presentations, and Other Accomplishments:

Abuel-Atta, A.A., DeSantis, M., and Wong, A. Encapsulated sensory receptors within intraorbital skeletal muscles of a camel. Anatomical Record, (in press).

DeSantis, M., Abuel-Atta, A.A., and Wong, A.M. Occurrence of muscle spindles and tendon organs in extraocular muscles of Camelus dromedarius. Neurosci Abs, 22, 2036 (1996).

DeSantis, M.E. Development of sensory receptors in skeletal muscle. NASA-Ames Research Center Cooperative Agreement NCC2-862. U.S. 1996.

DeSantis, M.E. Encapsulated sensory receptors in skeletal muscles: lessons from the dromedary camel and space rats. Seminar, School of Dentistry, Chulalongkorn University, Bangkok, Thailand, October 8, 1996.

DeSantis, M.E. Escapsulated muscle receptors: searching for answers in sundry places. Colloquium, Department of Biological Sciences, University of Idaho. Moscow, Idaho, September 20, 1996.

DeSantis, M.E. Rat neurological mutant - project development. University of Idaho Travel Grant. 1996.

DeSantis, M.E. Vestibular system structure in adult rats after their prenatal development in a near-zero gravity condition. NASA Idaho Space Grant Consortium, 1996.

DeSantis, M., Helmick, C., and Wong, A. Proprioceptors in skeletal muscle of rats that developed *in utero* aboard the space shuttle Atlantis. J. Idaho Acad. of Sci., (in press).

Hines and DeSantis, M. Size of vestibular nuclei in rats after prenatal development in microgravity. J. Idaho Acad. of Sci., (in press).

Genetically Engineered Plant Biomonitors in Microgravity

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Funding:

Project Identification:

Solicitation: 93-OLMSA-05

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $ 125,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: PGIM-01 (TBD)

Responsible NASA Center: KSC

Flight Hardware Required: PGU

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.

In 1996, the TAGES biomonitor plants were carried aloft on the KC-135 research aircraft out of Johnson Space Center. Plants experiencing parabolas exhibited marked expression of the reporter gene, indicating the onset of aberrations similar to those reported from shuttle misions. In fact, the degree of induction of reporter gene activity was actually higher than that reported by Dr. Musgrave for alcohol dehydrogenase induction during shuttle flights (Portfield et al., in press). The most dramatic expression of the reporter occurred in plants subjected to two flights in a single day. Plants from the double flights were ultimately exposed to 80 parabolas of altered gravity. The localization and intensity of the GUS reporter gene in the TAGES plants exposed to parabolic flights or those maintained as ground controls was determined by direct staining of intact plants. Quanitative analysis of reporter expression in flight and ground control plants was determined from frozen material recovered after the flights and is expressed as the total reporter gene activity per milligram protein recovered from the root.

Given the positive results obtained from the parabolic flights, a series of centrifuge experiments was conducted in order to rule out the periods of 2-G as possible causes of the activation of the stress response. TAGES plants were subjected to a 1- to 2-G centrifugation series which mimicked the hyper-G portion of the parabolic flights. TAGES plants were subjected to 80 truncated "parabolas," with each truncated parabola going from 1-G to 2-G over a time frame similar to that of the KC-135 recovering from a dive, while resting at 1-G for the time that

would be microgravity on the KC-135. The centrifuged plants demonstrated no visual or quantitative differences from the plants maintained as controls. In addition, all of the centrifuge plants, both experimentals and controls, had quanitative reporter activities similar to the ground controls from the KC-135 flights. These results offer additional proof of the concept of the original proposal: that reporter genes can be used to monitor the wide-range of alterations that spaceflight experiences impart on plants. In addition, these results show that at least some of the early stages of the alterations that occur in spaceflight can be examined in atmospheric parabolic flights and centrifugation series. The ultimate goal is to use this information to help design growing systems and media that reduce or eliminate stress responses.

Like all living organisms, plants constantly monitor their environment and make adjustments to their physiology as environmental needs dictate. Changes in environmental conditions almost uniformly lead to changes in gene regulation in plants, and these regulatory adjustments provide the altered molecular condition within the plant cell that allows the plant to survive and even grow in the new environmental situation. For example, plants exposed to microgravity conditions have shown ultrastructural characteristics that are similar to terrestrial plants exposed to hypoxia, but it is unknown whether the plant is actually responding to reduced oxygen potential or some secondary effect of microgravity. This research is dedicated toward the engineering of plants that are capable of reporting their perception of potentially adverse environmental situations to the investigator. Data from these plants as well as the entire experimental approach might also be used to examine the cause of certain plant growth anomalies that have resulted from exposure to adverse environmental conditions on Earth. Effective evaluation and dissection of plant genes, together with the development of tailored reporter gene systems, can provide the scientific community with plants capable of monitoring the growth conditions actually perceived by plants in adverse environments on Earth or in space.

FY96 Publications, Presentations, and Other Accomplishments:

Billups, A. Plant growth study: IFASs first foray into space. Explore, 1, 4 (1996).

Evaluation of Thermoregulation During Short-Duration Space Flight

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John Greenleaf; NASA Ames Research Center
Richard Gonzalez; U.S. Army Res. Instit. Environ. Med.
Stuart M.C. Lee; KRUG Life Sciences, Inc.

Funding:

Project Identification:
Solicitation: 95-OLMSA-01
Initial Funding Date:
Expiration:
FY 1996 Funding: $
Students Funded Under Research: 0

Flight Information:

Flight Assignment: ETSF (TBD)
Responsible NASA Center: JSC

Task Description:

The purpose of this study is to evaluate thermoregulatory responses of shuttle crew members before, during, and immediately after spaceflight. This flight investigation is currently in the queue awaiting a flight opportunity to be manifested as a small payloads study.

In 1996, we began ground-based definition studies to further validate the use of the telemetry pill system during bedrest and to evaluate changes in thermoregulation during microgravity simulation. This study will determine whether greater heat storage during exercise after simulated space flight (bedrest) is due to impaired skin vasodilation or impaired sweating. Before and immediately after 13 days of bedrest, 8 subjects will perform a submaximal, supine exercise test (20 min. each at 40 and 65% pre-bedrest VO2max) with measurements of core temperature (esophageal and gut temperatures), skin temperatures (thermistors), skin perfusion (laser Doppler flowmeter and forearm strain gauge), and local chest sweating (dew point hygrometry). Changes in thermal responses will be related to changes in blood volume during the bedrest, as measured via isotope dilution (125I labeled human serum albumin and 51Cr labeled sodium chromate).

In 1996, we also began the ground-based definition study employing a protocol nearly identical to the flight proposal. Nine subjects began the bedrest study. One subject dropped out prior to beginning bedrest and one subject dropped out after 12 days of bedrest because of a personal emergency at home. Seven subjects have completed all pre- and post-bedrest testing at this time. In 1997 we plan to finish the study and begin testing an additional two subjects.

The results of this study will have direct benefits to assessing the potential for heat-induced injuries for crew members during flight and recovery from flight. Significant Earth-based benefits may be realized by application of the non-invasive methodology used in this study to measure body temperatures. The miniatured telemetry system used in this study is being upgraded from currently available commercial systems. The flight-qualified system will be small and easier to use and much less susceptible to data dropouts due to the poor design of the original antenna system and to susceptibility of the receiver to electromagnetic interference. Small, portable "heat strain" indictors have direct application to conditions in which humans must work in extreme environments or while wearing heat-impermeable clothing: firefighters, soldiers in chemical warfare garments, workers in nuclear/chemical protection garments, etc.

Effects of Weightlessness on Vestibular Development [in Rat Pups]

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Funding:

Project Identification: 106-50-06
Solicitation: 93-OLMSA-03
Initial Funding Date: 7/94
Expiration: 6/97
FY 1996 Funding: $0
Students Funded Under Research:

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)
Responsible NASA Center: ARC

Task Description:

The lack of gravity is known to alter vestibular responses in developing and adult vertebrates. One cause of these altered responses may be changes in the connections between the vestibular receptor and the brain. Therefore, we propose to investigate the effects of gravity on the formations of connections between the gravity receptors of the ear and the brain in rat pups raised in space beginning at an age before these connections are made until near the time of birth, when they are to some extent functional. This investigation will make use of a novel technique, the diffusion of a lipophilic dye, DiI, in fixed tissue. This technique can be used to analyze the connections in specimens fixed immediately after landing of the space shuttle, thus minimizing changes due to the Earth's gravity. The evaluation of the data will enable us to detect gross deviations from normal patterns as well as detailed quantitative deviations.

Thus far, we have studied about two thirds of the flown embryos. Our data clearly indicate several effects of microgravity on neuronal development, two of which were already published as abstracts. The third, major effect, needs further confirmation using remaining flight and control material.

Research to date has found that: (1) the absence of gravity affects the rate of maturation of the gravistatic projections to the brain: The saccular and vestibular connections appear less mature in the flight animals than in the synchronous controls; (2) in control animals some ganglion cells in the geniculate ganglion project to the inner ear but many more do so in the flight animals; and (3) in both normal and flight animals efferent fibers have branches to both the saccule and the cochlea. The occurrence of multiple branches has not previously been reported in normal rats and was an unexpected finding.

As a result of these findings, we are now seeking answers for two new questions: A) is the projection of the non-gravistatic vestibular receptors of flight animals not only as mature as but even ahead of the control animals? In other words, is there a reciprocal effect of microgravity on the gravistatic versus non-gravistatic projections of the vestibular system?; and B) what is the degree of maturation of synaptic contacts between vestibular fibers and second-order neurons?

Thus far, we have found that there are fewer mature synaptic contacts between the saccular fibers and the vestibular nuclei in microgravity-exposed animals. In contrast, the semicircular canal afferents show no qualitative differences between flight and control animals. We have also calculated the total numbers of synapses in vestibular nucleus areas in flight and control animals, and our data thus far tend to show fewer synapses in flight animals.

The results of our research should allow us to obtain answers to the more general question, namely, whether there is a critical period during which the gravistatic and non-gravistatic components of the vestibular system compete for the targets in the vestibular nuclei as demonstrated in other maturing sensory systems.

Our data have opened an exciting new avenue of research into the anatomical basis of microgravity-related orientation deficits. Our study examined fetuses exposed to microgravity during a period when the vestibular system is just beginning to function. Our results show that fetuses exposed to microgravity have less mature gravity-sensitive projections compared to normal fetuses. Similarly, behavioral studies show that their littermates have similar behavioral deficits, and furthermore regained partial or complete responsiveness to gravity over time. We predict that these flight-induced anatomical alterations will be age related, and will be more pronounced and possibly permanent in animals exposed to microgravity soon after birth. Further anatomical and behavioral experiments are necessary to identify a possible critical period where gravity may be essential for the development of normal vestibular connections in neonatal rats.

If our continuing analyses support this hypothesis, we hope to identify a developmental period during which some gravistatic stimulation must be provided to ensure the development of proper connections between the gravistatic receptors in the ear and the vestibular nuclei in the brain.

FY96 Publications, Presentations, and Other Accomplishments:

Bianchi, L.M., Conover, J.C., Fritzsch, B., De Chiara, T., Lindsay, R.M., and Yancopoulos, G.D. Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. Development, 122, 1965-1973 (1996).

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Fritzsch, B. and Hallbook, F. A simple and reliable technique to combine oligonucleotide probe *in situ* hybridization with neuronal tract tracing in vertebrate embryos. Biotech. & Histochem., 71, 289-294 (1996).

Fritzsch, B., Nichols, D.H., Echelard, Y., and McMahon, A.P. The development of midbrain and anterior hindbrain ocular motorneurons in normal and in *Wnt*-1 knockout mice. J. Neurobio., 27, 457-569 (1995).

Fritzsch, B., Silos-Santiago, I., Smeyne, D., Fagan, A., and Barbacid, R., Reduction and loss of inner ear innervation in *trk*B and *trk*C receptor knockout mice: A whole mount Dil and SEM analysis. Auditory Neurosci., 1, 401-417 (1995).

Frizsch, B. The afferent innervation of the ear: Only two neurotrophins and their receptors are necessary to maintain it. Promega NeuroReport, 1, 11-13 (1996).

Hellmann, B. and Fritzsch, B. Neuroanatomical and histochemical evidence for the presence of common lateral line and inner ear efferents and of efferents to the basilar papilla in a frog, *Xeonpus laevis*. Brain, Behav. & Evol., 47, 185-194 (1996).

Norgren, R.B., Gao, C., Green, E., Ji, Y., and Fritzsch, B. Tangential migration of LHRH neurons in the medial telencephalon in association with transient axons extending from the olfactory nerve. Neurosci. Letters, 202, 9-12 (1995).

Rosa-Molinar, E., Fritzsch, B., and Hendricks, S.E. Organization-activational concept revisited: Sexual differentiation in an atherinomorph teleost. Hormones & Behav., 30, 563-575 (1996).

Effect of Spaceflight on the Development of the Circadian Timing System

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Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $215,000

Joint Agency Participation: NIH

Solicitation: 93-OLMSA-03

Expiration: 9/96

Students Funded Under Research: 10

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

Responsible NASA Center: ARC

Task Description:

Animals have evolved and developed within the constant gravitational environment of the Earth and the dynamic circadian changes in the environment associated with the 24-hour day. The circadian timing system (CTS) is an important temporal organizer controlling both the physiology and behavior of organisms. For example, conditions, such as jet lag, shift work, and some sleep and mental disorders are frequently associated with dysfunction of the CTS. Our previous studies have shown that exposure of both mature and developing animals to hyperdynamic fields via centrifugation significantly affects the CTS. In addition, mature animals exposed to the microgravity environment of space flight exhibit altered CTS function. Although previous studies have demonstrated that exposure to space flight during the prenatal period can significantly delay a few general parameters of development, it is not known whether prenatal exposure to space flight will significantly alter maturation of the central nervous system, physiology, and behavior. This research will begin to examine the anatomy and physiology of the CTS of animals exposed to space flight during the prenatal period. These studies will focus on four areas: (1) the laminar development of the retina which provides visual pathway to the CTS; (2) the development of soma size and oxidative metabolism of neurons within the suprachiasmatic nucleus (SCN), the circadian pacemaker of the CTS; (3) the development of photic responsiveness of the SCN; and (4) the development of temperature and activity rhythms to examine the onset and maturation of circadian function. The retina and CTS provide excellent models for central nervous system development due to their well-characterized neural development and regulatory function in physiology and behavior.

During the fiscal year 1996 of the NASA Grant "Effect of Spaceflight on the Development of the Circadian Timing System," several of the critical tasks have been accomplished. During this time, we have been processing the retinal and brain tissue. We have beem analyzing this tissue in order to determine the effect of prenatal exposure to space flight on the development of the Circadian Timing System.

We successfully received the cross-fostered rat pups along with their foster dams and siblings from each of the four experimental groups (Flight, Flight Delayed Synchronous, Vivarium, and Vivarium no laparotomy). All experimental pups were implanted at PN21 with an intraperitoneal abdominal transmitter. Each pup was studied

until PN90. Temperature and activity data were collected at 5-minute intervals, and body mass was determined twice per week. All pups exhibited stable, low-amplitude rhythms of temperature and activity immediately upon recovery from surgery (PN21). The rhythms gradually increased in amplitude until PN40-50, at which time stable, mature rhythms were seen. There were no significant differences between the groups in terms of rhythm development.

This research examines the anatomy and physiology of the CTS of animals exposed to space flight. These studies may be useful for understanding the effect of an environmental stressor on prenatal development. These results may be usefully applied to many conditions where stress during pregnancy can affect the developing fetus.

Effects of Hypogravity on Osteoblast Differentiation

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Funding:

Project Identification:

Initial Funding Date: 2/94

FY 1996 Funding: $37,943

Joint Agency Participation: NIH

Solicitation: 93-OLMSA-04

Expiration: 6/96

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-C1 (STS-59, 4/94) and NIH-C3 (STS-63, 2/95)

Responsible NASA Center: ARC

Flight Hardware Required: ST1

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 that of 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 majority of the cartridges Analysis of spent media samples revealed that the cultures acquired contamination at the time of transfer from ground-based Cellco. units to the STL hardware. In general, light and electron microscopy did not

show any significant differences in cell morphology between flight and ground control groups and showed little evidence of collagen accumulation in either flight or ground-control cultures. Northern analysis revealed that the cells expressed significant levels of mRNA for osteopontin as well as osteocalcin, which is a later marker of osteoblast differentiation. Thus, the cells appeared to differentiate to a limited extent in the course of the experiment despite the contamination. Significant differentces were not observed in the amounts of glucose consumed and lactate produced between flight and ground control samples at the end of the flight. However, given the problem of contamination, data acquired from this flight are not informative, and conclusions about the effects of flight cannot be drawn.

Results from this project require confirmation in additional flight experiments. Future experiments should address the questions of whether microgravity alters energy metabolism as well as the differentiation of cultured osteoblasts. To adequately answer these questions will require the use of both on-board centrifuge controls as well as methods to accurately quantify cell number in each sample.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Doty, S.B., Boskey, A., Binderman, I., Globus, R.K., and Holton, E.M. The effect of spaceflight on bone and cartilage cell differention. 5th International Conference on Mineralized Tissues. October 22, 1995.

Effects of Altered Gravity on the Photosynthetic Apparatus

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Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $97,273

Joint Agency Participation: NSAU

Solicitation:

Expiration: 9/96

Students Funded Under Research: 5

Flight Information:

Flight Assignment: CUE (STS-87, 11/97)

Responsible NASA Center: KSC

Flight Hardware Required: PGF

Task Description:

Photosynthesis is the single most important contributor to food and fiber production, and is susceptible to a variety of stresses which can limit plant yields. This process must play a crucial role in a closed renewable life support systems during long spaceflight missions, for food, atmosphere, and water regeneration, and it will be critical to understand the effects of the space environment on the dynamics of such a complex process. Space missions in the past have provided tantalizing, albeit often anecdotal, glimpses of the effects of microgravity on cellular morphology and cell division and elongation, and how these effects may impact photosynthetic physiology. Differences in cell size, shape, division and elongation rates have been noted, as well as effects on respiration rate and on differentiation and cellular development, such as on plastid distribution and structure.

At present there are only few data concerning structure and functioning of chloroplasts in microgravity, and they are often contradictory. Space-grown tissues often show a decrease in thylakoid membrane stacking, and an increased number of plastoglobuli, indicative of thylakoid membrane turnover. Reports of the pigment content of space-grown tissues are contradictory, with some groups demonstrating an increased chlorophyll content in space-grown pea, while others suggest the opposite. Both chlorophyll and carotenoid levels of maize were reduced after growth for 19 d on Mir, and levels of pigment were reduced 35 to 50% in *Chlorella*. Kordyum and colleagues have suggested that changes in membrane structure may in part account for altered plant cell morphology during space flight. Alterations in membrane fluidity, mediated by changes in fatty acid composition or in the level of stress-inducted lipid peroxidation, could have a profound effect on resource partitioning across membranes and mechanisms of chemiosmotic energy conservation. In recent work, Tripathy and coworkers monitored several aspects of photosynthetic function from wheat grown in microgravity. They found decreases in light-driven electron transport capacity of 25% at saturating light intensities.

It will be important to examine both the **light harvesting/energy conversion** aspects of photosynthesis which occur on thylakoid membranes of the chloroplast, and **carbon fixation pathways,** the enzymes of

which are found in the stroma, in understanding spaceflight effects on this process. Our understanding of thylakoid membrane structure and function has advanced greatly in the last ten years because of a concerted research effort encompassing molecular biology techniques, the biochemical isolation of membrane protein complexes, and the three dimensional structural analysis of reaction center protein crystals with atomic resolution. The elegance of these molecular approaches are now available to aid in our understanding of the impact of microgravity on photosynthesis.

A "tissue sharing" experiment, in which we will examine the leaves of *Brassica rapa* plants for their photosynthetic characteristics, has been developed for the PGF flight hardware. These plants will also be used in the primary pollination and fertilization experiment, and choice of plant materials and growing conditions were optimized for the primary experiment. In this experiment, plants will be germinated on orbit, and will be harvested at various timepoints to provide: (1) leaf material which has been fixed during space flight, (2) leaf material which has been placed in the GN2 freezer and returned to Earth in a frozen state, and (3) leaf material which as been returned to Earth as fresh tissue. These tissues will permit us to use a suite of techniques to examine the photosynthetic physiology of space-grown plants, including biophysical (fluorescence spectroscopy and induction kinetics), biochemical (functional assays of Rubisco and electron transport capacities, lipid/protein interactions visualized by chlorophyll/protein assemblages, and pigment and protein compositional analyses), and ultrastructural (TEM), enhanced by immunogold labelling of specific polypeptides) measurements. In particular, we will monitor those parameters which are indicative of the mechanisms of stress impacts on photosynthetic physiology.

Work to date has been focused on defining the PGF growth conditions necessary for *Brassica rapa*, consistent with the goals and objectives of both the pollination and the photosynthesis experiments. The strategy which was selected involved the use of florists foam as a nutrient storage substratum while maintaining seeds in germination envelopes made of germination paper. Half-strength Hoaglands solution was selected as the nutrient solution. These experiments were performed in anticipation of an October 1996, SVT.

Further work has defined the electrophoresis and electron transport characteristics of thylakoid obtained from *Brassica rapa* green tissue.

Photosynthesis is the single biological process for the energetic capture of sunlight and its storage in chemical form. As such, it is critical for food and fiber production, and the understanding of this basic process is necessary for the development of strategies for sustainable and ecologically sound systems of agricultural production. Considerations of photosynthetic efficiency are especially important, since this process utilizes carbon dioxide as a substrate. Global climate changes are predicted as a result of fossil fuel-induced increases in atmospheric carbon dioxide, and this is also a characteristic of the atmosphere aboard the shuttle.

FY96 Publications, Presentations, and Other Accomplishments:

Armbrust, T.S., Chitnis, P.R., and Guikema, J.A. Organization of photosystem I polypeptides examined by chemical crosslinking. Pl. Phys., 111, 1307-1312 (1996).

Brown, C.S., Hilaire, E.M., Guikema, J.A., Piatuch, W.C., Johnson, C.F., Stryjewski, E.C., Peterson, B., and Vordermark, D.S. Metabolism, ultrastructure, and growth of soybean seedlings in microgravity: Results from the BRIC-01 and BRIC-03 experiments. American Society for Gravitational and Space Biology annual meeting, October 1995, Washington, D.C.

Hilaire, E., Paulsen, A.Q., Brown, C.S., and Guikema, J.A. Plastid distribution in columella cells of a starchless *Arabidopsis* grown in microgravity (STS-63). American Society for Gravitational and Space Biology annual meeting, October 1995, Washington, D.C.

Hilaire, E., Peterson, B.V., Guikema, J.A., and Brown, C.S. Clinorotation affects morphology and ethylene production in soybean seedlings. Pl. & Cell Phys., 37, 929-934 (1996).

Juergensmeyer, M.A., Odom, W.R., and Guikema, J.A. Examination of the plastid-cytoskeleton interface using immological probes. American Society for Gravitational and Space Biology annual meeting, October 1995, Washington, D.C.

Application of Physical and Biological Techniques in the Study of the Gravisensing and Response System of Plants

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 95-OLMSA-01

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $9,798

Students Funded Under Research: 1

Flight Information:

Flight Assignment: MFA-1

Responsible NASA Center: KSC

Flight Hardware Required: MFA

Task Description:

The gravisensing system, specifically the role of amyloplasts, will be studied by applying directional stimuli using high gradient magnetic fields (HGMF) which enable the displacement of amyloplasts. As has been demonstrated in previous work, HGMF exert a directional, repulsive force on diamagnetic substances such as the starch in amyloplasts. We have shown that *in vitro* and *in vivo* amyloplasts move along the gradient of the magnetic field. Space research will establish threshold levels of the magnetic field strength required for root curvature. Aside from studying the application of HGMF in directional growth control, the experiments will test whether the force exerted by amyloplasts or their position inside sensory cells controls the direction of growth. The graviresponse system and root growth in general will be studied by investigating the behavior of the plant cytoskeleton in ground and microgravity conditions. We will use microgravity-grown, fixed, and HGMF-manipulated roots to establish the organization of the cytoskeleton and whether it is affected by microgravity. Other experiments are designed to test if differential growth in microgravity leads to the same organization of the cytoskeleton (microtubules and actin filaments) that are observed on Earth. Typically, elongation of cells is characterized by deposition of cellulose microfibrils that are usually aligned perpendicular to the direction of growth. This is thought to establish the preferential direction of elongation of cells and organs. The proposed research will provide insight in the fundamental organization and operation of the graviresponse system of plants and whether only sensory cells or relatively large areas of the plant need directional stimulation to establish normal growth.

The objective of the previous research was a detailed analysis of curvature induction in roots and intracellular magnetophoresis of amyloplasts. Based on the information from these studies, we examined whether positively gravitropic shoot curvature could be induced by high gradient magnetic fields (HGMF), an objective that had been attempted earlier but was not achieved presumably because of insufficiently small magnetic gradients.

We tested coleoptiles (oat, *Avena sativa*, and barley, *Hordeum vulgare*) by positioning them in a HGMF. The dynamic factor H2/2 of the field was 109 to 1010 Oe2/cm. The HGMF was generated by inserting a ferromagnetic wedge into a uniform magnetic field (ca. 4.5 kOe). To minimize gravity effects, the seedlings and magnets were rotated on a 1-rpm clinostat. After 4 hours 90% of coleoptiles had curved toward the denser

HGMF, (i.e., behaved as predicted for negatively gravitropic organs). Coleoptiles in a magnetic field next to a non-ferromagnetic wedge (i.e., no HGMF), showed no preferential curvature. The small size of the area of non-uniformity of the HGMF allowed mapping of the sensitivity of the coleoptiles by varying the initial position of the wedge relative to the coleoptile apex. When the ferromagnetic wedge was placed lower than 1 mm below the coleoptile tip, only 58% of the coleoptiles curved toward the wedge indicating that the cells most sensitive to intracellular displacement of amyloplasts and thus gravity sensing are confined to the top one mm portion of barley coleoptiles. Similar experiments with tomato (*Lycopersicum esculentum*) hypocotyls also resulted in curvature toward the HGMF.

Based on these and previous results we now have strong support for the amyloplast-based gravity sensing system in higher plants. A second accomplishment is the usability of HGMF to substitute gravity in roots and shoots under microgravity conditions. Future investigations, partially dependent on a shuttle experiment scheduled for 1998, focus on several objectives: Does microgravity alter the density of starch and do plants acquire higher or lower gravi- or magnetophoretic sensitivity.

The observation that amyloplasts in the periphery of the root cap cells do not sediment upon gravistimulation or move due to magnetophoretic forces suggests that the cytoskeleton fixes these organelles in place. We will study the magnitude of the forces exerted by the cytoskeleton and which component (actin filaments or microtubules) contributes to the anchoring of amyloplasts and possibly other organelles.

The application of high gradient magnetic fields to investigate the gravity sensing mechanism of plants has wide implications on two levels. First, the research utilizes and improves a novel mechanism of intracellular displacement of starch-filled amyloplasts and the resulting growth response of plants. Secondly, the growth response is likely to be tightly linked to the perception of a stimulus analogous to gravity. Therefore the research addresses the larger problem of studying the signal perception/response mechanism in plants. Such studies will generally promote our understanding of plant growth regulation. In particular, the high gradient magnetic field-dependent growth response will elucidate the change in elongation growth and thus directional deposition of cell wall material biomass. In addition, as with all basic research, an improved understanding of basic growth phenomena will have important implications for improving growth, biomass production on Earth, and better understanding of the bio-mechanic properties of growing plants and thus will benefit the average citizen.

FY96 Publications, Presentations, and Other Accomplishments:

Hasenstein, K.H., Kuznetsov, O.A., and Blancaflor, E.B. Induction of plant curvature by magnetophoresis and cytoskeletal changes during root graviresponse. Proceedings of the 6th European Symposium On Life Science Research In Space, ESA SP-390, 71-74 (1996).

Kuznetsov, O.A. and Hasenstein, K.H. Magnetophoretic induction of root curvature. Planta, 198, 87-94 (1996).

Wan, Y. and Hasenstein, K.H. Preparation and characterization of anti-idiotypic antibody to probe putative abscisic acid receptors. Int. J. BioChrom., 2, 77-78 (1996).

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 1996 Funding: $

Students Funded Under Research: 2

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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 that 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 terrestial 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 as 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 highly 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.

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 1996 Funding: $ 10,747

Students Funded Under Research:

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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 all of the histomorphometric analysis. We have characterized the changes caused by space flight to tendon attachments to the tibia, fibula, and femur. We have determined the effects of space flight on the calcaneus and 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 develoment 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 bone but a rat's metabolism is much more rapid than that of a human, if the osteoporosis occurred in rats during a 11 day flight, it could occur in humans experiencing a longer (3 month) space flight. There was evidence of microfractures in the tibia of space flight rats, suggesting that bones weakened by osteoporosis during space flight may fracture on return to Earth. This event could disable astronauts on their return to gravity. The results of this study also suggested that loading bones weakened by osteoporosis will not promote healing, but will likely result in fracture.

FY96 Publications, Presentations, and Other Accomplishments:

Johnson, R.B. and Abel, S.E. Comparison of effects of spaceflight on hindlimb and forelimb bones. ASGSB Bull.., 10, 59 (1996).

Johnson, R.B., Tsao, A.K., St. John, K.R., Betcher, R.A., Tucci, M.A., Parsell, D., and Benghuzzi, H.A. Effect of spaceflight on the structure of the fibula. FASEB J., 10, A753 (1996).

Johnson, R.B., Tsao, A.K., St. John, K.R., Betcher, R.A., Tucci, M., Parsell, D.E., and Benghuzzi, H.A. Atrophy of the fibula occurs coincident to spaceflight. J. MS Acad. Sci., 41, 61 (1996).

Parsell, D.E., Barkley, P.J., Tsao, A.K., St. John, K.R., Betcher, R.A., and Johnson, R.B. Effects of spaceflight on rat femoral density. J. MS Acad. Sci., 41, 63 (1996).

Plant Embryos and Fidelity of Cell Division in Space

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93-OLMSA-05

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $81,000

Students Funded Under Research: 2

Flight Information:

Flight Assignment: BRIC-08 (STS-78, 7/96)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

Task Description:

The study 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; and
- Ground controls will be developed in parallel to the flight experiment.

Hypothesis: I have proposed that the chromosomal and nuclear abnormalities encountered in various plants exposed to space are due to a combination of factors including the biological status of the systems and the way in which they are grown, exposed to, and ultimately, the way in which they experience multiple stresses. The extent to which space-specific changes become manifest is dependent on the extent of pre-existing stresses in the system. This has been suggested in a variety of plant species grown in space but has been particularly amenable to study using our *in vitro* developing daylily embryoid system. The following summary hypothesis based on flight observations allows us to harmonize disparate results from several space experiments: (a) the more completely developed a system, the less likely it is to show cell stress during growth; the less morphologically complex, the greater the vulnerability; (b) the "size/packaging" of the genome (karyotype) are also significant experimental variables; plants with larger genomes (e.g., polyploids) seem to be more space-stress tolerant; and (c) a single space-associated stress is inadequate to produce a significant adverse response unless the stress is severe or a biological parameter necessary to "amplify" it exists. On this view, an appropriate "stress match" with other non-equilibrium determinants, much like a "tug of war," can result in genomic insults being manifested in space-grown materials.

The general question being asked is: Can altered mitosis and chromosome behavior in developing plant cells predictably be adversely modified by the space environment by modifying the experimental set-up to deliberately achieve a non-optimized environment? The more specific question is: Can adverse alterations in osmotic status and water relations (water stress) pre-dispose cells to become damaged cytologically in the space environment?

Task: To utilize adverse water relations as the prime "stressor" in combination with biological status of materials being exposed to the stress in order to assess whether water can serve as one of the putative stresses. Ground work has been carried out to allow water-stressed cells grown on media of differing gel strength and "dryness" or "wetness" to be followed and subjected to chromosomal analysis. Gel wetness and dryness is achieved by curing of the agar for varying periods of time, as well as by adjusting the mix of agar and gel rite, gellan gum. Task: To design a match of developmental stage and level of potential vulnerability so as to run the experiment(s) with samples that are "more" or "less" able to compensate for the anticipated insults of space flight. (In addition to daylily embryoids being clonal, there is the potential for utilizing "phenocritically sensitive or labile" stages that indicate particularly vulnerable developmental stages. Simply put, the earlier the stage, the more demanding of a rigorously-maintained and -controlled environment the cells are. We predict that there is likely to be more damage in these kinds of cells in space since we cannot supra-optimize their growth conditions. Conversely, the less phenocritically-sensitive the stage, the less demanding of a rigorously-controlled environment and the less damage expected).

Experiments were made to evaluate different fractions of cultures to be exposed to space. Evaluations were made by exposing filtered fractions between specific size ranges to the medium for development. Larger materials were determined as being more tolerant of stress; smaller less resistant. Task: Development of chromosome methods to ensure that data could be obtained in the event space flight conditions were to unexpectedly adverse that cell division might be temporarily "shut down" and not allow data to be immediately obtained upon return to Earth. Samples were run at elevated temperatures—up to 34 degrees—for three weeks. Techniques needed to obtain quality chromosome preparations under adverse conditions were worked out by resuscitating materials by exposing them for brief periods to fresh medium prior to fixation. Cell division resumes and allows cytological analyses. While levels of chromosomal damage may be slightly lower than might be observed on immediate analysis, a 2 to 3 day "freshening" of the exposed embryos does not obviate picking up aberrant patterns. Task: To run the experiment in BRIC 08. Materials were tracked every other day on Earth to assess the level of embryo growth and stability of the genome. Post-flight or Analysis after Recovery: Chromosome analysis was carried out on Earth at Stony Brook after recovery by examining materials fixed with mitotic arresting agent (colchicine) as well as direct fixations. These analyses relied mainly on squashes of randomly selected embryos pulled off their support surfaces in the petri dishes. Structure of chromosomes was assessed by cutting and matching homologs. No operations were performed in space. Task: Assessment of Embryogenic Response. Somatic embryo production was scored at the end of the experiment after petri dish inoculation and at weekly intervals in the controls up to 4 weeks. A positive score for somatic embryogenesis consisted of recognition of various stages of somatic embryogenesis characteristic for daylily. Germination of somatic embryos and rearing them to rooted plantlets *in vitro* was done for some samples by transferring randomly selected embryos of different stages of development to appropriate media.

Plants are important from many viewpoints. From the time we get up in the morning and brush our teeth with toothpaste thickened with plant extracts and flavored with peppermint oil, to the food we eat, to the garments we wear, to much of the furniture we use, to the cotton pillow and sheets we lie down on at bedtime, our daily lives are intimately affected by plants.

Throughout the complex process of plant growth, a series of well-orchestrated and coordinated events ranging from cell division to differentiation must occur. Indeed, under field conditions, if the needed cell biological and biochemical and molecular events that ultimately give rise to resultant form and function are not properly mobilized and realized both temporally and spatially, the plant will lose out in the competition with better "designed" plants. At the laboratory level, major differences in structure, metabolism, biochemistry, and ultrastructural architecture will be apparent. There is strong evidence that gravity plays a major role in directing

the way in which plant cells "orchestrate" these required events in their zones of cell division, differentiation, and maturation. Developing somatic embryos of daylily, *Hemerocallis*, provides an excellent model system that will allow us to test how the cell biological, biochemical, and physical events leading to the formation of both specialized and unspecialized types of cells comprising the plant embryo are uncoupled, or mobilized and modulated during growth in a long-duration microgravity environment. An experiment has been designed to provide detailed insights into these important processes by examining growth and cytological and biochemical performance in daylily embryocytes in specially contrived configurations designed to reveal special responses at the level of morphology, histology, cell biology, ultrastructure, biochemistry, and chemistry. Development and responses in protracted microgravity will be looked at from the perspective of the level and precision of cell division in critical growing regions of embryo shoot and root apex as they respond to alterations in their loading-bearing capabilities, mitotic errors that might occur during differentiation and specialized cell and tissue development in the novel environment of microgravity, and the level of activity of the genome in terms of special gene expression in expected and unexpected ways. Efforts will be made to characterize changes in the cell cycle and potential modifications to it as a consequence of adaptation or response to development in microgravity as compared to normal development at l-G. Any changes detected in altered growth characteristics or disturbed development in microgravity will be correlated at the cell, tissue, and organ levels and further detailed at the biochemical and molecular levels. Application of a series of cell biological and biochemical techniques will be brought to bear to explain the mechanism(s) of responses that we expect to detect at the level of cells, tissues, organs, and the entire embryo. A major effort will be directed towards determining unequivocally whether observed perturbations to the daylily system are due to microgravity proper or whether they are due to indirect effects of the space environment. Accordingly, this project recognizes that in order to obtain reliable data in this important experiment, considerable effort has to be expended through pre-flight ground studies to ensure that as rigorously controlled an environment will be attainable for the performance of the experiment.

To the experimental biologist, plants are very interesting since they have evolved mechanisms that enable them to sense and use various environmental signals and messages to their advantage in the course of their lives. Because plants are generally immobile, they have to deal with situations as they arise—they cannot "run away." Plants are very well adapted to using "information" from the outside world. This means that an understanding of how plant growth control is achieved is very important.

Space flight experiments show that metabolism, productivity, and specialization characteristics of a variety of plant cells are altered. The study of these reactions to space has led to a better understanding of the ways plant cells, especially cultured embryogenic cells, grow and the mechanisms by which such cells develop and control production of cell components which are important in agriculture.

More specifically, our research has been concerned with how growth and development of embryos and plantlets from non-sexual (body) cells is affected and controlled by gravity and the space environment. Cloning experiments have been carried out on Earth and in space to generate embryos from embryocytes (also known as embryo initials) and the work indicates that there is significant impact on embryo differentiation and growth. Studies on the nature of shape and form, genetic changes and how they occur and are modulated has shed significant light on the process of regeneration from cultured plant cells. This information is critical to much plant biotechnology since the field relies on manipulating, changing, and managing developing plant cells and regenerating and cloning plantlets from them. One of the current and major constraints to reliably controlling genetic engineering in plants for overall improvement purposes is the lack of a full understanding of the controls mechanisms as free cells develop into embryos and from these plants. The information gained from Earth and in space has pointed the way to exploring the control mechanisms more effectively.

It is clear that the intimate and adaptive, even evolutionarily controlled, relations between the atmosphere, the soil, and the growing plant that are normally achieved on Earth will not be easily duplicated in space. A somewhat empirical approach appears justified based on our limited knowledge of space flight environments and the responses of plants in those environments. Clearly more experimental data need to be obtained under well-defined conditions.

In this context, it is important to recognize that modern plant science and agricultural engineering have produced some remarkable advances, but none of these would have been possible without the ability to build on an ancient foundation. A similar foundation is not available for those wishing to grow plants in space. We therefore will only be able to make progress if we have a body of data on which we can build.

As real progress is made towards adding reliable baseline data to our store of knowledge, and the growing of plants in space becomes increasingly reliable, it is certain that many members of the scientific community will become attracted to carrying out space biology experiments. As it now stands, limitations in our ability to grow plant materials reliably have prevented a broad plant biological sciences community from becoming more involved. I believe that it is in the best interest of science and NASA not to minimize the constraints to growing plants reliably in space.

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Molecular and Cellular Analysis of Space Flown Myoblasts

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Project Identification:

Solicitation: 93-OLMSA-04

Initial Funding Date: 4/94

Expiration: 4/96

FY 1996 Funding: $54,000

Students Funded Under Research: 4

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-C1 (STS-59, 4/94) and NIH-C3 (STS-63, 2/95)

Responsible NASA Center: ARC

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.

In 1996, we expanded our investigation into the effects of space flight on muscle differentiation by utilizing the well-characterized cell line of L8 rat myoblasts. Having flown L8 cells successfully on space shuttle mission STS-45 and, most recently, STS-63, reculturing of these cells on Earth resulted in their unexpected decreased ability to fuse and differentiate into myotubes. Therefore, we decided to compare the presence of several important structural, muscle cytoskeletal proteins (sarcomeric myosin, developmental myosin, myosin heavy chain-fast, titin, and desmin) in the space-flown cells to that of the ground control cells. The expressions of these proteins are known to be highly regulated during normal myoblast fusion and terminal differentiation.

Spaceflown (L8SF), ground control (L8GC) and stock control (L8AT) cells were grown on gelatin-coated coverslips in 24-well plates at 37°C. At subconfluency, confluency, and confluency + 4 days, cell monolayers were screened using a standard indirect immunofluorescent staining procedure employing monoclonal antibodies to these cytoskeletal proteins. The secondary antibody was FITC-labeled goat-anti-mouse antibody. Under normal growth conditions at confluency + 4 days, L8 rat myoblasts begin to fuse and form large multinucleated myotubes. Our results for the L8AT, L8GC and L8SF cells stained at confluency + 4 days for each of the five muscle cytoskeletal proteins are as follows:

A. MYOSIN

Myosin, the most abundant myofibrillar protein of the thick filament, was investigated as three discrete types including 1) sarcomeric myosin, 2) developmental myosin, and 3) myosin fast type heavy chain. The immunofluorescent staining in the L8AT and L8GC cells for all three types of myosin appeared as expected at confluency + 4 days. Large, distinct and long flowing fibers of each individual protein were observed within the myoblasts after they had fused to form multinucleated cells containing 20-30 nuclei (MN30 cells). In the more advanced stages of fusion, initial formation of the sarcomeric bands could readily be observed for sarcomeric and developmental myosin. However, the space-flown cells yielded strikingly different yet varying results. The L8SF cells from STS-45 displayed two diverse staining patterns: 1) individual, non-fusing L8SF cells exhibited a diffuse, irregular, and non-specific staining (smear-like) across the monolayer of myoblasts, and 2) minimal cell fusion where multi-nucleated cells containing only 2-3 nuclei (MN3 cells) were detected with little evidence of differentiation toward the formation of myotubes. The L8SF cells from STS-63, however, while still not exhibiting a significant extent of overall fusion, seemed to advance slightly further into their differentiation and developmental stage. Visible staining patterns due to the immunofluorescence included 1) well-defined, individual non-fusing cells; 2) fusion to form MN3 cells; and, in addition, 3) multinucleated cells containing 6-8 nuclei (MN8 cells) with the initial stages of myotube formation. Here, it is important to note that even though the myotubes were beginning to form in the L8SFs from STS-63, it was quite evident that the stage of maturation of these myotubes, when compared to controls, was not as advanced. In fact, when the L8SF cells from STS-63 were grown up and continuously observed until confluency + 8 days, the myotube formation had not progressed any further than the formation seen at confluency + 4 days.

B. TITIN

Although titin is not as abundant as myosin in the thick filament, it plays an important structural role in the development of the sarcomere. Similar to the staining pattern results of the myosin proteins, the L8AT and L8GC cells presented clear, well-defined MN30 cells along with the initial formation of easily identifiable myotubes. However, the quantity of these titin stained myotubes was not as numerous as those of myosin. The L8SF cells from STS-45 also displayed similar staining patterns to that of myosin: 1) individual, non-fusing L8SF cells revealed a diffuse, irregular and non-specific staining pattern throughout the monolayer of myoblasts, and 2) MN3 cells were detected with no differentiation toward myotube formation. In contrast to myosin, the titin staining patterns for the L8SF cells from STS-63 revealed only one specific staining pattern: a sharp, clear staining of MN3 cells with no myotube differentiation as of the current investigation.

C. **DESMIN**

Desmin serves as a connective Z-line protein in the overall structure of the myofibrils and is known to appear earlier in myogenesis than titin. The L8AT and L8GC cells staining for desmin were few but extremely clear and evident. The staining patterns for these control cells appeared as 1) individually stained cells, and 2) as MN3 cells beginning myotube formation as some have developed long, thin spindle-like ends. The staining patterns for the L8SF cells from STS-45 resulted in observations that were consistent with those previously documented for myosin and titin: 1) individual, non-fusing L8SF cells in a diffuse, non-specific staining pattern, and 2) MN3 cells. However, in addition to these two previously documented patterns, a significant third staining pattern was observed. MN3 cells which had begun to develop long, thin spindle-like ends were observed in the L8SF cells corresponding to the myotube formation seen in the control cells. Again, it is important to note that these MN3 cells with myotube formation were not as abundant as the number seen in the control cells. The immunofluorescent staining procedures for the L8SF cells from STS-63 are in progress.

All data to this point indicate that flying L8 rat myoblasts on the space shuttle for a duration of 7 to 10 days at subconfluent densities results in significant phenotypic alterations in cellular protein structure of the myoblast cells: 1) non-fusion of space-flown cells, 2) minimal fusion of space-flown cells to form MN3 cells with no myotube formation, 3) minimal fusion of space-flown cells to form MN3 cells with rudimentary stages of myotube formation and 4) fusion of MN8 cells with the initiation of myotube formation. Although specific protein alterations differed slightly between cells from the two flights, the most notable phenotypic alterations in cell differentiation were consistent among all the spaceflown L8 myoblast cells when compared to the controls: 1) overall decrease in cell fusion, 2) fusion of cells resulted in less dense multi-nucleated cells, and 3) a significantly lower level of differentiation in myotube formation.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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An Experiment to Study the Role of Gravity in the Development of the Optic Nerve

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Project Identification: RF-65

Solicitation: 93-OLMSA-03

Initial Funding Date: 5/94

Expiration: 5/96

FY 1996 Funding: $82,283

Students Funded Under Research: 3

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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.

During this fiscal year, all the data for this experiment was analyzed. This included data from the brain and retinas of 10 flight and 11 control animals.

Following landing, the control and flight rat pups were divided into groups corresponding to developmental time points of postnatal days p2, p14, p21, and p47. An injection into the superior colliculus of each animal at the appropriate time point. The animals were sacrificed two days later in-order to (1) measure the volume of dye actually delivered into the superior colliculus and (2) to measure the area of staining in the corresponding retina due to the retrograde uptake of the dye by axons.

During FY96, we performed extensive image processing to quantitate the results of above experiment. Photomicrographs of the retinas were digitized, and each ganglion cell on these photomicrographs was hand tagged. Using a moment generating algorithm, parameters which defined a 2D gaussian distribution of these cells was determined for each animal. The area of an elliptically shaped gaussian contour enclosing 60% of the stained population was defined as the "projected area stained." This area was computed and then normalized by the area of the entire retina for each animal.

Sequential epifluorescence and bright field photomicrographs of vibratome sections of the brain of each animal were digitally combined to determine the area of dye falling within the superior colliculus. The volume stained

was computed for each animal and was normalized by the total volume of the superior colliculus. Six views of each rendered brain were generated to qualitatively evaluate the location and depth of each injection.

A metric called retinotopic magnification was defined to describe the changes in mapping from axonal branches in the superior colliculus to ganglion cells in the retina. The metric was designed to measure changes in retina-brain connectivity while being somewhat invariant to growth.

We defined retinotopic magnification as: R = Normalized area stained of retina/ Normalized Vol stained of Sup. colliculus.

Our findings suggest a normal reduction in retinotopic magnification. The P47 magnifications are elevated due to a slightly higher cell counting threshold being applied by the technician analyzing this group of photomicrographs.

In previous experiments, retinotopic magnification of 14 day old rats was shown to be no different than 2 day old rats. A significant drop occurs in retinotopic magnification of the 21 day old animals. However, rats normally open their eyes at 14 or 15 days. Inexplicably, in the present experiment, all rats opened their eyes at 13 days of age. It is possible that this earlier eye opening led to maturation of the visual system by the time they were studied on day 14.

These results show, as expected, a trend toward less magnification with time as the visual system matures. The trend is toward a one to one mapping from a small region in the superior colliculus to a focal projection in the visual field. These trends are seen in both flight and control groups. Differences between the flight and control groups were not statistically significant. It remains uncertain whether normal post-natal maturation of the central nervous system could occur if the rats has continued to be reared in microgravity.

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.

The techniques developed in quantitating retinotopic projections may help evaluate the efficacy of gene therapy interventions for developmental diseases of the optic nerve, such as optic nerve hypoplasia. These techniques allow such treatments to be developed and tested in a mathematically rigorous manner.

Influence of Space Flight on Bone Cell Cultures

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Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1996 Funding: $102,173

Solicitation: 93 OLMSA-04

Expiration: 12/95

Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-C1 (STS-59, 4/94) and NIH-C3 (STS-63, 2/95)

Responsible NASA Center: ARC

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.

Analysis is now in progress to identify and localize proteins of interest at the ultra structural level in the flight, basal, and control groups of bone cells prepared for STS-63. This is being accomplished by immunocytochemical means with cell cultures chemically fixed, embedded, and sectioned for electron microscopy. Osteopontin and bone sialoprotein have been found in the extracellular matrices of the respective culture groups, and their presence correlates with other molecular and biochemical studies reported earlier. The immunochemical characterization of collagen, osteocalcin, fibronectin and other proteins important in mineralization events will follow.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Gerstenfeld, L.C., Zurakowski, D., Schaffer, J.L., Nichols, D.P., Toma, C.D., Broess, M., Bruder, S.P., and Caplan, A.I. Variable hormone responsiveness of osteoblast populations isolated at different stages of embryogenesis and its relationship to the osteogenic lineage. Endocrinology, 137, 3957-3968 (1996).

Landis, W.J. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. Bone, 16, 533-544 (1995).

Landis, W.J., Hodgens, K.J., Arena, J., Song, M.J., and McEwen, B.F. The structural relation between collagen and mineral in bone as determined by high voltage electron microscopic tomography. Microscopy Res. & Technique, 33, 192-202 (1996).

Landis, W.J., Hodgens, K.J., Song, M.J., Arena, J., Kiyonaga, S., Marko, M., and McEwen, B.F. Mineralization of collagen occurs on fibril surfaces: Evidence from conventional and high voltage electron microscopy and three-dimensional imaging. J. Struct. Biol., 117, 24-35 (1996).

Meazzini, M.C., Schaffer, J.L., Toma, C.D., Gray, M.L., and Gerstenfeld, L.C. (abstract) Osteoblast cytoskeletal modulation in response to a spatially uniform biaxial mechanical strain *in vitro*. Transactions of the Orthopedic Research Society, 20, 87 (1995).

Winnard, R.G., Gerstenfeld, L.C., Toma, C.D., and Franceschi, R.T. Fibronectin gene expression, synthesis, and accumulation during *in vitro* differentiation of chicken osteoblasts. J. Bone & Mineral Res., 10, 1969-1977 (1995).

Yang, R. and Gerstenfeld, L.C. Signal transduction pathways mediating parathyroid hormone stimulation of bone sialoprotein gene expression in osteoblasts. J. Biol. Chem., 271, 29839-29846 (1996).

Effects of Microgravity on Pathogenesis and Defense Responses in Soybean Tissues

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Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $

Joint Agency Participation: NSAU

Solicitation:

Expiration: 9/96

Students Funded Under Research: 6

Flight Information:

Flight Assignment: CUE (STS-87, 11/97)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

Task Description:

Anecdotal evidence suggests plants grown in microgravity are more susceptible to microbial invasion. If true, the implications for crop production in space are serious: the increased accessibility of plants to microorganisms may mean that the plants are not only more susceptible to recognized pathogens, but they may also be susceptible to pathogenic colonization by *opportunistic* pathogens, i.e., organisms that are not normally pathogens to the plant. The project defined here brings the expertise of molecular and cellular biologists from the Ukraine (O. Nedukha, V. Prima, and E. Kordyum) and the USA (J. Leach and J. Guikema, KSU; C. Brown, KSC) together to explore how microgravity affects plant susceptibility. For the proposed studies, we have selected the interaction between *Phytophthora sojae* and soybean as a model system. *P. sojae* causes root and stem rot of soybean. Our reasons for selecting this disease interaction are the following: (1) *P. sojae* is a devastating pathogen of soybean causing annual yield losses exceeding $250 million; (2) two of our collaborators (Guikema and Brown) have extensive experience with microgravity-induced alterations in soybean physiology; (3) resistant interactions between *P. sojae* and soybean roots do not require light as do foliar pathogens; in the BRIC hardware where our experiments will take place, there is no light; (4) the genetics of the interactions between different soybean cultivars and different races of the fungus are well documented; (5) the molecular mechanisms of resistant interactions between *P. sojae* and soybean are well characterized; and (6) the experimental system will provide sufficient amounts of space-grown tissues for the proposed analyses by both Ukrainian and USA scientists.

Our first objective is to quantitatively measure the effects of microgravity on susceptibility of soybean seedlings to root rot caused by *Phytophthora sojae*. Previous observations that plants grown in microgravity are more susceptible than unit gravity-grown plants have not been quantitative. Thus, this objective is important because as we begin to dissect out which factors contribute to susceptibility and to what extent each contributes, we need an established baseline of quantitative data for microgravity-induced susceptibility.

Increased susceptibility may have several underlying causes; these will be explored in our second objective. Microbial vigor may be increased in microgravity or the microbes may become somewhat resistant to the effects of toxic compounds produced by the plant. Alternatively, changes in the plant cellular structure or the cells ability to respond to pathogens may result from growth in microgravity. In this project, we will emphasize the plant side of the interaction. We will apply concepts derived from the current understanding of how plants resist pathogens, targeting specific flaws that might allow enhanced susceptibility. Enhanced susceptibility in microgravity might result from changes in the *performed* plant barriers to pathogen ingress (such as decreased wall strength resulting from reduced lignification) or changes in nutrient partitioning (such as increased carbon availability or membrane fluidity). **Our Ukrainian collaborators will compare cell wall structure and plasma membrane structure and fluidity in soybean seedlings grown in microgravity versus unit gravity and determine if these correlate with increased susceptibility to the root rot pathogen, *Phytophthora sojae*. C. Brown will determine if carbohydrate partitioning in the seedlings is altered in microgravity, and if this correlates with susceptibility.**

Plant development in microgravity also might impair the plant's ability to *actively* resist pathogen ingress, that is, *induced* plant defense compounds may not be formed or, if formed, may not locate to appropriate sites. In general, compounds that are induced during plant resistance responses include soluble antibiotic compounds called phytoalexins, structural reinforcements such as the phenolic polymers lignin or suberin, enzymes such as those involved in phytoalexin biosynthesis or phenolic polymer deposition (e.g., peroxidases), or enzymes that may directly affect the pathogen, such as chitinases or B-glucanases. **We will determine if, after growth in microgravity, differences in the accumulation and distribution of structural compounds (phenolic polymers) and enzymes whose accumulation and location is correlated with resistance (anionic peroxidases and phospholipase D) occur in soybean challenged with two races of *P. sojae*, race 1 and 25, which cause resistant and susceptible responses, respectively, under conditions of unit gravity.**

A. Adaptation of Assay for BRICs.

An assay has been established where disease development can be quantitatively monitored in space-grown soybean. The assay involves a quantitative assessment of the development of soybean roots and lateral roots and of pathogen colonization of root tissues in uninfected or infected plants grown at unit and microgravity. For both flight and ground experiments, soybean seeds (cv Williams 82, Rps k1) are placed in sterile plastic seed growth pouches with an inner germination paper sleeve. Seeds are oriented with the embryonic root tip pointing toward the bottom of the pouch; each pouch is divided into four chambers and contains four seeds. The germination paper is moistened with a dilute fungal growth medium (lima bean medium). For infected seedlings, fungal inoculum (*P. sojae*, race 1 or 25) is added to each pouch. The pouches are placed inside BRIC canisters and stowed in the middeck locker. At various times during flight, pouches are removed and photographed on a grid (for measurement of root growth and lateral root development) and then root tissues are removed and either subjected to fixation or frozen (for microscopic analysis of tissue invasion by fungal hyphae). Under laboratory conditions, during resistant interactions the average root length is shorter and the number of lateral roots formed are fewer than in susceptible interactions.

B. Science Verification Test

In October '96, a Science Verification Test (SVT) was performed at KSC using this setup. Microscopic examination of roots fixed during the SVT revealed *P. sojae* oospores in the susceptible tissues but not in the resistant tissues by day 8. Roots of soybean exposed to fungi in resistant (race 1) and susceptible (race 25) interactions were shorter than the control (untreated) roots. In addition, fewer laterals were formed on soybean roots after exposure to *P. sojae* than on untreated roots. However, neither root lengths nor the numbers of laterals formed varied significantly between the two fungal treatments. In fact, root growth during the SVT in all treatments was significantly greater than that observed in laboratory controls. One environmental factor that

differed between the SVT samples and laboratory controls was that CO_2 levels were greater than 10 times ambient conditions. Experiments are underway to determine if CO_2 concentrations affect root growth and pathogenesis. If so, a means to control CO_2 concentrations will need to be implemented. Preliminary experiments demonstrate that increased aeration of samples reduces the increased root growth attributed to CO_2 effects. Additional modifications implemented after the SVT include a simplification in the sampling procedures, and a new design for the apparatus for fixation of samples.

C. Evaluation of defense response compounds.

Tissues from unit and microgravity experiments will be evaluated for changes in several defense compounds. Initially, our focus is on the deposition of wall-associated barriers (phenolic polymers) and the accumulation of two defense-associated enzyme groups: anionic peroxidases and phospholipase D (PLD). Anionic peroxidases may be involved in wall strengthening; PLD may be involved in membrane degradation as well as cell signal transduction. Changes in anionic peroxidase and PLD accumulation and location will be determined by electron microscopy of immunogold labeled tissues. Antisera to PLD is available; antisera to the peroxidases will be made. Changes in deposition of phenolic polymers will be evaluated histochemically.

The studies proposed will reveal whether growth in microgravity renders soybean plants more susceptible to ingress by a root fungal pathogen, in general, and if microgravity compromises the plant's ability to mount a resistance response. Based on our current understanding of the mechanisms by which plants defend themselves from pathogens, and what is currently known about morphological changes in plant cells grown in microgravity, we have predicted a number of potential "flaws" in the host defensive system. The experiments here will provide correlative evidence if some of these predictions are correct or probable. Furthermore, we will gain an understanding of the effects of microgravity on protein distribution in and around plant cells. The comparisons of resistant and susceptible host/pathogen interactions are important because it is possible that impaired development of a performed barrier, such as reduced lignification of the cell wall, may "override" any induced defense responses. Although reasonably well-characterized biochemically, most events associated with defense in unit gravity are correlative. The unique parameter in our experiments as compared to past studies of host plant resistance is the microgravity environment; we predict that comparisons of resistant and susceptible responses in unit vs. microgravity will provide new and important clues as to which events correlated with resistance are truly involved in host defenses. Finally, *P. sojae* is a devastating pathogen of soybean, causing annual yield losses exceeding $250 million; thus, any information that might provide clues to control the disease will be of major benefit to production of the crop.

FY96 Publications, Presentations, and Other Accomplishments:

Ardales, E., Leung, H., Vera Cruz, C.M., Mew, T.W., Leach, J.E., and Nelson, R.J. Hierarchical analysis of spatial variation of the rice bacterial blight pathogen across diverse agroecosystems in the Philippines. Phytopath., 86, 241-252 (1996).

Leach, J.E. "Induction of defense responses in rice" in "Molecular Aspects of Pathogenicity and Host Resistance: Requirements for Signal Transduction," Mills, D. and Kunoh, H. (eds). Academic Press, NY, 115-128 (1996).

Leach, J.E. Genes and proteins involved in avirulence and aggressiveness of *Xanthomonas oryzae* pv. oryzae to rice. 8th International Congress - Molecular Plant-Microbe Interactions, Knoxville, TN. July 17, 1996.

Leach, J.E. Physiology and molecular biology of resistance in bacterial blight of rice. Department of Plant Pathology, University of Missouri, Columbia, MO. April 24, 1996.

Leach, J.E. Molecular interaction between *Xanthomonas oryzae* pv. oryzae and rice. Department of Plant Pathology, Washington State University, Pullman, WA. February 29, 1996.

Leach, J.E. Molecular interactions between *Xanthomonas oryzae* pv. oryzae and rice. Department of Microbiology, University of Washington, Seattle, WA. February 27, 1996.

Leach, J.E. and White, F.F. Bacterial avirulence genes. Ann. Rev. Phytopathol., 34, 153-179 (1996).

Leach, J.E., Zhu, W., Chittoor, J.M., Ponciano, G., Young, S.A., and White, F.F. "Genes and proteins involved in aggressiveness and avirulence of *Xanthomonas oryzae* pv. oryzae to rice" in "Advances in Molecular Genetics of Plant-Microbe Interactions," Stacey, G., Mullin, B., and Gressholf, P. (eds). APS Press, Minneapolis, MN, 191-198 (1996).

Vera Cruz, C.M., Ardales, E.Y., Skinner, D.Z., Talag, J., Nelson, R.J., Louws, F.J., Leung, H., Mew, T.W., and Leach, J.E. Measurement of haplotypic variation in *Xanthomonas oryzae* pv. oryzae within a single field by Rep-PCR and RFLP analyses. Phytopath., 86, 1352-1359 (1996).

Young, S.A., Wang, X., and Leach, J.E. Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with *Xanthomonas oryzae* pv. oryzae. Pl. Cell, 8, 1079-1090 (1996).

Effects of Micro-G on Gene Expression in Higher Plants

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 95-OLMSA-02

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $47,000

Students Funded Under Research:

Flight Information:

Flight Assignment: BRIC-09 (STS-80) and BRIC-10 (STS-85, 7/97)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

Task Description:

Although various effects of microgravity at the whole plant, organ, tissue, and cellular levels have been observed, little is known about the molecular mechanisms involved. The PI proposes to use the BRIC hardware to conduct two experiments which may yield significant results about alterations in gene expression in space-grown seedlings. The first experiment is to use transgenic plants that express GH3 promoter-GUS and SAUR promoter-GUS fusion genes to study the effects of microgravity on expression of auxin- and gravistimulation-inducible genes, GH3 and SAUR, at the organ and tissue levels. This study should not only provide information about how expression of the auxin- and gravistimulation-inducible genes is altered at microgravity as compared to the ground controls (1-G and clinorotation), but also enable us to gain some insights into the effects of microgravity on auxin transport/distribution and/or tissue's sensitivity to auxin in higher plants. The second experiment is to identify and clone genes whose expression is altered under the microgravity environment using a differential cDNA library screening method or RNA Differential Display technique. Molecular cloning and characterization of gravity-regulated genes is a crucial step to elucidate the mechanisms of the gravity effects on growth, developmental, and metabolic processes in higher plants.

An experiment was recently (November 19, 1996, STS-80) conducted to study the effects of microgravity on the expression of auxin-regulated GH3 and SAUR genes in transgenic tomato and tobacco seedlings that express the auxin-inducible GH3 gene promoter-GUS and SAUR gene promoter-GUS fusion genes. Preliminary results indicate that the expression of both the GH3 promoter-GUS and SAUR promoter-GUS fusion genes was significantly enhanced (2 to 5 fold increases) in space-grown seedlings when compared with the ground control seedlings. The GH3 promoter-GUS gene was activated in cotyledons and elongating regions of hypocotyls and roots of the space-grown seedlings. The expression of the SAUR promoter-GUS gene in the elongating region of hypocotyls in the space-grown seedlings was significantly higher than in that of the ground controls. In addition, the SAUR promoter-GUS gene was activated in the roots of the space-grown seedlings while the expression of the SAUR promoter-GUS gene was not detectable in the roots of the ground controls. Furthermore, a dramatic reduction in root growth and a significant increase in hypocotyl length were observed in the space-grown seedlings, which is well correlated with the activation pattern of the auxin regulated genes. Currently, the PI and co-workers are characterizing the effects of auxin and inhibitors for auxin transport and

ethylene biosynthesis on the GH3 promoter-GUS and SAUR promoter-GUS gene expression, and conducting microscopic analysis of morphological changes of the space-grown tobacco and tomato seedlings.

Gravity has a variety of effects on the structure and function of plants identifiable at the whole plant, tissue, and cellular levels. The gravitropic response of higher plants makes roots grow downward into soil for absorbing water and mineral nutrients and shoots grow upward for harvesting maximum light energy for photosynthesis which is crucial for maximum productivity of crop plants. The proposed research to examine the effects of microgravity on the expression of auxin-inducible genes (auxin is an important hormone involved in the gravitropic response and growth/development of higher plants) and to clone/characterize gravity-regulated genes should provide some insights into the molecular mechanisms of the plant tropic response and the effects of gravity on plant growth and development. Thus, the proposed research should yield a new understanding of the effects of gravity on higher plants and may provide a basis for improvement of growth rate of higher plants grown in space. The latter is an important step toward the commercial application of space using plants as bio-reactors and the development of bio-regenerative life support systems to support crews in extra-terrestrial environments.

FY96 Publications, Presentations, and Other Accomplishments:

U. S. Patent #: [Undetermined] Li, Yi. Production of seedless fruits via a gene transfer approach. (in progress)

Strabala, T.J., Wu, Y., and Li, Y. Combinatorial effects of cytokinin and auxin transport inhibitors: Alteration of organogenesis and organ development from the shoot apical meristem. Pl. & Cell Phys., 37 (8), 1178-1182 (1996).

Osteoblast Adhesion and Phenotype in Microgravity

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Funding:

Project Identification:

Solicitation: 93-OLMSA-04

Initial Funding Date: 1/95

Expiration: 12/95

FY 1996 Funding: $27,924

Students Funded Under Research:

Joint Agency Participation: NIH

Flight Information:

Flight Assignment: NIH-C4 (STS-69, 7/95) and NIH-C6 (STS-80, 11/96)

Responsible NASA Center: ARC

Task Description:

Bone loss during space flight is well documented, but remains incompletely understood. Among the unanswered issues are the direct effects which microgravity exerts on bone cells, and the mechanisms by which these cells recognize changes in gravity. This study will focus on bone cells of the osteoblast family, which synthesize bone matrix and may also participate in its breakdown (resorption) by regulating the formation and activity of bone-resorbing cells, osteoclasts. The experiment will test the hypothesis that microgravity can produce direct effects on osteoblastic cells similar to those of regulatory hormones. In addition, the study will examine whether microgravity alters the interaction of osteoblastic cells with their matrix resulting in changes in shape or cellular organization known to affect cell function.

In this study, cells will be cultured in the mid-deck compartment of the space shuttle in the Space Tissue Loss (STL) 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 during FY 96 focused on evaluation of samples obtained from STS-69 and preparation for STS-80. Results from STS-69 indicated that ROS 17/2.8 cells were metabolically active during the experiment. Both the flight samples and ground controls released lactate into the culture medium as well as alkaline phosphatase, the paracrine factor prostaglandin E2, and the intracellular second messenger cyclic AMP. Ground samples tended to exhibit slightly higher PGE levels and lower cAMP levels than flight samples, but there was substantial variability and the pattern was not completely consistent.

In both ground and flight cartridges the number of cells seen on the Cytodex beads was noticeably lower than in samples collected before the flight at the time beads were loaded into cartridges. This suggested that a number of

cells had detached from the beads during the course of the experiment. Cells from the experiment also appeared to lack a well-organized cytoskeleton, in contrast to cells on Cytodex beads under pre-experimental conditions. Thus it was difficult to obtain reliable morphological data. As a result of this finding, however, a modification was made in the protocol for subsequent experiments. Cytodex-3 (collagen-coated) beads were replaced with Biosilon beads made of tissue culture-treated plastic. The Biosilon provides a more effective surface for the attachment of cells as well as adhesive molecules produced by cells or present in serum. This change was implemented on the STS-80 flight.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Bourghol, A., Einhorn, T.A., Yamazaki, M., Atkinson, G.M., and Majeska, R.J. Fibronectin expression during fracture healing in the rat. Trans. Orthop. Res. Soc. 21:618, poster presentation, ORS 42nd Annual Meeting, Atlanta, GA, 1996.

Kagel, E.M., Majeska, R.J., and Einhorn, T.A. Effects of diabetes and steroids on disc fracture healing. Curr. Opin. in Orthopaedics , V6, 7-13 (1995).

Majeska, R.J. "Culture of osteoblastic cells" in "Principles of Bone Biology." Edited by: Bilezikian, J.P., Raisz, L.G., and Rodan, G.A. Academic Press, NY, pp 1229-1237, 1994.

Majeska, R.J. Making and breaking bone: The osteoblast's perspective. Oregon Health Sciences University, March, 1996.

Majeska, R.J. Provisional matrices in fracture healing. University of Connecticut Health Center, April, 1996.

Yamazaki, M., Majeska, R.J., Goto, S., Moriya, H., and Einhorn, T.A. Localization of pro-alpha à2(V) collagen transcripts during fracture healing. Trans. Orthop. Res. Soc. 21:202, podium presentation, ORS 42nd Annual Meeting, Atlanta, GA. 1996.

Ca^{2+} Metabolism and Vascular Function After Space Flight

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Funding:

Project Identification:

Solicitation: AO-93-OLMSA-01

Initial Funding Date: 7/95

Expiration: 12/95

FY 1996 Funding: $

Students Funded Under Research:

Flight Information:

Flight Assignment: NIH-R4 (STS-85, 1997)

Responsible NASA Center: ARC

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 principal aim of NIH.R4 was to determine the influence of dietary calcium and microgravity on calcium metabolism, blood pressure regulation, and vascular function in the spontaneously hypertensive rat (SHR). Beginning at 3 weeks of age and continuing throughout the experiment, the SHR were maintained on either high (2.0%) or low (0.2%) calcium diets. At 7 weeks of age the two diet groups (n = 7/diet) were flown on the Space Shuttle Columbia. Two ground control groups were included in the experiments completed at Kennedy Space Center. One was a genetic control for the spontaneously hypertensive rat, the Wistar-Kyoto rat, and the other was a maturational control. Both control groups were fed diets identical to the flight group.

On recovery from the space shuttle, conscious blood pressure measurements were completed within 6 hours of landing using an indirect tail-cuff method. Direct recordings of mean arterial pressure were subsequently made from the carotid artery while the animals were anesthetized with halothane (2% in O_2) prior to blood sampling. Direct blood pressure measurements were completed within 17 hours of landing. A number of additional end points were measured on each of the rats including mesenteric resistance artery function, platelet intracellular calcium at rest and following agonist stimulation, serum levels of calcium regulating hormones, whole blood ionized calcium and serum electrolytes, bone mineralization, and calcium binding proteins (calmodulin and calbindin D9K).

The results showed that blood pressure was altered by both diet and microgravity but there was no interaction between the two variables. Blood pressure was significantly higher in animals on low calcium diets than animals on high calcium diets throughout the experiments. Blood pressure was somewhat lower in the conscious flight animals after landing than in the SHR control group ($p = .053$). However, after being anesthetized, blood pressure in the flight group was, on average, 18 mmHg higher than in the SHR control group ($p < .0001$) regardless of dietary condition.

As with blood pressure, there were substantial changes in vascular function in the flight animals independent of the dietary condition. Overall, the mesenteric vasculature was less responsive in the flight animals than the control animals to all stimuli. There was less contraction to norepinephrine and less relaxation to acetylcholine. There was no difference in the response to sodium nitroprusside. The net result of these changes on total peripheral resistance is unknown. While it might be assumed that the reduction in maximal contraction to norepinephrine would be indicative of a probable reduction in vascular resistance, the apparent dysregulation of endothelial function and the subsequent ability to relax the vessels to the extent observed in normal animals argues in the other direction. That is, the vascular response to the many vasodilatory signals that impinge on the vessel may be more important in determining vascular tone than the response to norepinephrine. Impaired vascular relaxation would lead to an elevation of total peripheral resistance and may have contributed to the shift in blood pressure regulation in the flight animals. The presence of significant correlations between mean arterial pressure and vasodilation in our data but not between norepinephrine induced contractions and mean arterial pressure is indicative of the importance of vascular relaxation in determining blood pressure.

The vascular data are of paramount importance to the issue of orthostatic intolerance. Failure of the vasculature to respond appropriately to either vasoconstrictors or vasodilators limits the potential for hemodynamic adjustments and may underlie, in part, the orthostatic intolerance that has been reported in astronauts on return to Earth's gravity. Limited vascular responses may be inadequate to compensate for the reductions in stroke volume that occur post-space flight.

Along with the changes in blood pressure regulation and vascular function, there were alterations in subcellular calcium handling. Basal platelet intracellular free calcium was higher in animals on low calcium diets. Likewise, calcium storage, as indicated by peak calcium release to the calcium ionophore ionomycin, was elevated in animals fed low calcium diets. Space flight animals had lower levels of basal and stimulated intracellular free calcium. Intracellular calcium levels were closely associated with vascular relaxation. How the change in intracellular calcium in the space flight animals influenced the pattern of blood pressure responses is currently under investigation.

Systemic calcium metabolism was also altered by space flight. Whole blood ionized calcium was predictably elevated by microgravity. Parathyroid hormone was elevated in the space flight rats but 1,25(OH)2D3 was not elevated. The changes related to microgravity conditions were comparable across diet groups such that differences that existed on the diets in the ground controls were apparent in the flight rats as well. We are currently analyzing this data to determine their relationship to blood pressure and vascular function. One preliminary finding indicates the responsiveness of the vessels to agonists was intimately related to variations in calcium metabolism, particularly ionized calcium levels and intracellular calcium storage.

To summarize the results, we have gathered important new information that suggests a change in blood pressure regulation following space flight and data suggesting that the change in regulation may be related to alterations in vascular function.

The research conducted on PARE-04 will benefit mankind in multiple ways. First and foremost, the research addresses the issue of essential hypertension, a disease that afflicts 40 million Americans and is a leading risk factor for strokes and heart attacks. In the past 10 years it has become apparent that calcium metabolism is closely linked to blood pressure regulation. In part, the link may be due to the role of calcium as a second messenger within cells. One manifestation of that role is increased vascular tone when intracellular calcium levels are high. Paradoxically, increasing the level of calcium available outside of the cell through increased dietary calcium reduces calcium levels inside the cell. This promotes vasorelaxation and lowers blood pressure. Understanding the pathways that link calcium as a nutrient that is ingested to calcium as a second messenger will help us understand the etiology of hypertension. The research that will be undertaken in PARE-04 will provide new perspectives on the role of calcium in blood pressure regulation. Zero gravity conditions place considerable strain on calcium metabolism because of unloading of the skeleton and loss of bone calcium. In the short term, resorption of calcium from the skeleton results in hypercalcemia, altered calcium regulating hormones, and reduced absorption of calcium. Ultimately, it leads to a depletion of calcium stores that may result in elevated blood pressure, osteoporosis, and other maladies associated with limited calcium availability.

In a sense, space flight is analogous to pregnancy where there is also a drain on calcium reserves as the fetus develops. During pregnancy, blood pressure is particularly sensitive to dietary calcium intake. Low levels of dietary intake are associated with elevated blood pressure and the development of gestational hypertension and preeclampsia. Supplemental dietary calcium, on the other hand, lowers blood pressure and reduces the risk of developing a hypertension disorder during pregnancy by two-thirds. Dietary calcium supplementation may prove to have a beneficial effect during space flight as well. The issue of dietary calcium and blood pressure regulation is one that 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 Earth-bound.

FY96 Publications, Presentations, and Other Accomplishments:

Brooks, V.L. and Hatton, D.C. Chronic infusion of angiotensin II resets baroreflex regulation of plasma norepinephrine and corticosterone concentration by an arterial pressure-independent mechanism. Am. J. Physiol, (in press).

Gaboury, C.G. and McCarron, D.A. Blood pressure and calcium-regulating hormones. Curr. Opin. Endocrinol. Diabetes, 3, 271-276 (1996).

Hatton, D.C. and McCarron, D.A. Dietary salt and hypertension. Curr. Opin. Nephrol. Hypertens., 5, 166-169 (1996).

Hatton , D.C., Brooks, V., Yue, Q., and McCarron, D.A. The cardiovascular response to stress: Baroreflex resetting and hemodynamics. Am. J. Physiol., (in press).

Hatton, D.C., Harrison-Hohner, J., Coste, S., Qi, Y., and McCarron, D.A. Effect of prenatal calcium supplementation on infant cardiovascular risk factors. Proceedings of Perinatal Biology, University of Hawaii (in press).

Hatton, D.C., Haynes, R.B., Oparil, S., Kris-Etherton, P., Pi-Sunyer, F.X., Resnick, L.M., Stern, J.S., Clark, S., McMahon, M., Morris, C., Metz, J., Ward, A., Holcomb, S., McCarron, D.A. Improved quality of life in patients with generalized cardiovascular metabolic disease on a prepared diet. Am. J. Clin. Nutr., 64, 935-943 (1996).

Hatton, D.C., Reusser, M.E., and McCarron, D.A. Salt and human health: The evidence against mandated restriction. Nutr. Rev., (in press).

McCarron, D.A. and Hatton, D. Dietary calcium and lower blood pressure - We can all benefit. JAMA, 275, 1128-1129 (1996).

McCarron, D.A. and Reusser, M.E. Body weight and blood pressure regulation. Am. J. Clin. Nutr. , 63, 423S-425S (1996).

McCarron, D.A., Oparil, S., and Resnick, L.M. Comprehensive nutrition plan improves cardiovascular risk factors in essential hypertension. Hypertension, (in press).

McCarron, D.A., Weder, A.B., Egan, B.M., Krishna, G.G., Morris, C.D., Cohen, M., and Oparil, S. Blood pressure and metabolic responses to moderate sodium restriction in Isradipine-treated hypertensive patients. Am. H. Hypertens., (in press).

Roullet, J.B., Xue, H., McDougal, P., Chapman, J., Roullet, C.M., and McCarron, D.A. Farnesyl analogues inhibit vasoconstriction in animal and human arteries. J. Clin. Invest., 97, 2384-2390 (1996).

Microgravity Effects on Pollination and Fertilization

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $146,000

Joint Agency Participation: NSAU

Solicitation:

Expiration: 9/96

Students Funded Under Research: 6

Flight Information:

Flight Assignment: CUE (STS-87, 11/97)

Responsible NASA Center: KSC

Flight Hardware Required: PGF

Task Description:

The conclusion of a third flight experiment on reproductive development in *Arabidopsis* in fall 1994 (STS-68) provided us with extensive material for analysis of seed formation during space flight. These seeds were prepared for microscopy and stained for cytochemical localization of seed storage reserves. When compared with ground controls, the material was found to be indistinguishable. Previous experiments on STS-54 and STS-51 with this same biological system had shown reproductive development to abort during space flight prior to seed formation. These experiments were conducted in closed chambers which probably leads to stagnant air layers around the plants in microgravity where convective air movement is lacking. On STS-68, an active airflow was provided, and all stages of reproductive development occurred normally on orbit. Since reproductive development thus seems to be possible in microgravity if proper environmental conditions are provided, we have sought a new experimental system that will allow us to closely compare the timing of developmental milestones in space flight and ground control material. This was not strictly possible in *Arabidopsis* because of its pollination biology. *Arabidopsis* is self-pollinating. For our new model system we sought a plant that requires pollen transfer from a different source (i.e. it is self-incompatible) in order to closely control the timing of pollen transfer to the stigma and therefore initiation of the fertilization sequence. *Brassica rapa* has been chosen for this experiment. *Brassica rapa* is closely related to *Arabidopsis*, however it bears larger flowers that can be manually pollinated using a pollination device. Through the Crucifer Genetics Cooperative, many different lines of rapid-cycling *Brassica rapa* are available, and line 1-59 is especially well-suited for space flight experiments because its short stature allows it to fit within the confines of a Plant Growth Chamber in the Plant Growth Facility flight hardware. Details of soil-less culture were worked out to allow an easy integration of 11-day old plants into PGCs prior to launch. In-flight procedures including labeling flowers, pollinating, and in-flight fixation procedures were determined. This effort is geared to the scheduled launch of an experiment on STS-87 as part of the CUE payload in October 1997.

Observations on reproductive material from STS-68 (Chromex-05) experiment were completed and a manuscript was prepared and submitted for publication on cytochemical localization of reserves in seeds of *Arabidopsis* that

had developed under space flight conditions. This was the first instance of completely normal seed development in microgravity. Analysis of roots from STS-54, STS-51 and STS-68 demonstrated that the space flight material's roots are consistently hypoxic compared to the ground controls. The decrease in oxygen availability to roots under space flight conditions was determined to be 28% in an agar system. Different ways of growing plants for short durations in the Plant Growth Facility were investigated and an adaptation of the phenolic foam method devised by Krikorian was adapted for use in growing *Brassica rapa* for the CUE experiment. Plastic sleeves surrounding the individual plant pairs allow for easy insertion of plants into the foam matrix just prior to launch and streamline the preparation of the Plant Growth Chambers. Pollination wands and bee sticks were evaluated as possible means for transferring and storing pollen on orbit. Bee sticks were found to collect about three times more pollen grains than the synthetic pollination wands and were therefore chosen to become part of the pollination kit. The kit was designed to contain bee sticks individually and provide desiccant protection of harvested pollen grains after pollination. The general experiment scenario was devised to meet the objective of closely comparing development that occurs on orbit and in 1-G when times after pollination are equal.

Understanding physical processes that contribute to the success or failure of plant reproduction will allow plant biologists to make further progress in tackling problems in plant pollination and fertilization under normal conditions. The microgravity environment of orbital platforms provides a unique mechanism for investigating these physical processes, since gravity-dependent processes are perturbed in ways that are not possible in a 1-G environment.

However, another way this project is of value is in the pride and excitement it stimulates. Interest in the space program is high, especially among children. I have found that the goodwill these experiments foster toward basic research in general is extremely important. Growing plants in space is something that excites the average person and heightens awareness of science in general. In 1997 the CUE will be featured in the news and will likely obtain national and international press coverage. My experiment is paired with an educational outreach component, CUE-TSIPS, which provides classroom simulation of the space flight experiment for children in the US and Ukraine. This is an unprecedented attempt to bring public involvement into a plant space biology experiment. When people think about why plants would be grown in space (i.e., to provide food and atmosphere cleansing for the crew members), they may remember and appreciate the importance of agriculture to sustaining their daily lives on earth.

FY96 Publications, Presentations, and Other Accomplishments:

Crispi, M. L., Porterfield, D.M., and Musgrave, M.E. (abstract) Control of growth and reproductive development in *Arabidopsis thaliana* by non-earthnormal metabolic gas ratios. ASGSB Bull., 9, 50 (1995).

Crispi, M.L., Porterfield, D.M., Murgia, M., and Musgrave, M.E. Role of metabolic gases in reproductive failure under spaceflight conditions: Ground based studies with *Arabidopsis*. SAE Technical Paper, Series # 961391, (1996).

Kuang, A., Musgrave, M.E., and Matthews, S.W. Modification of reproductive development in *Arabidopsis thaliana* under spaceflight conditions. Planta, 198, 588-594 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions. Ann. Bot., 78, 343-351 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. Dynamics of vegetative cytoplasm during generative cell formation and pollen maturation in *Arabidopsis thaliana*. Protoplasma, 194, 81-90 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. (abstract) Anatomy and cytochemical localization of reserves during seed development in *Arabidopsis* under spaceflight conditions. Pl. Phys. Suppl., 111, 73 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. (abstract) Seed development in *Arabidopsis* under spaceflight conditions. ASGSB Bull., 9, 54 (1995).

Musgrave, M.E., Kuang, A., Matthews, S.W., and Ramonell, K.M. (abstract) Plant reproduction under spaceflight conditions. ASGSB Bull., 9, 92 (1995).

Porterfield, D.M., Crispi, M.L., and Musgrave, M.E. (abstract) Metabolic responses of *Arabidopsis* to altered atmospheres. Pl. Phys. Suppl., 111, 71 (1996).

Porterfield, D.M., Matthews, S.W., and Musgrave, M.E. (abstract) Transcription, activity, and localization of alcohol dehydrogenase in the roots of *Arabidopsis thaliana* following exposure to spaceflight conditions. ASGSB Bull., 9, 16 (1995).

Xiao, Y., Kuang, A., Porterfield, D.M., and Musgrave, M.E. (abstract) Substrates for growth of *Brassica rapa* in the PGF and SVET. ASGSB Bull., 10, 40 (1996).

Effect of Microgravity on Bone Development

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Funding:

Project Identification:

Initial Funding Date: 1/95

FY 1996 Funding: $

Solicitation: 93-OLMSA-03

Expiration: 12/97

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NIH-R2 (STS-70, 6/95)

Responsible NASA Center: ARC

Task Description:

This project will study the expression of the tissue plasminogen activator and collagenase enzymes in fetal and postnatal rats exposed to microgravity during development. The findings of this research will throw light on the importance and role of gravity in developing bone.

Exposure to zero gravity has been shown to cause a decrease in bone formation. This implicates osteoblasts as the gravity-sensing cell in bone. Osteoblasts also are known to produce neural proteinases, including collagenase and tissue plasminogen activator (tPA), which are thought to be important in bone development and remodeling. The present study investigated the effects of zero gravity on development of calvariae and their expression of collagenase and tPA. After exposure, *in utero*, to zero gravity for nine days on the NASA STS-70 space shuttle mission, the calvariae of rat pups were examined for the presence and location of these two proteinases by immunohistochemistry. The pups were from gestational day 20 (G20) to postnatal (PN) day 35 in age. Both collagenase and tPA were found to be present at all ages examined, with the greatest amount of both proteinases present in the PN 14 rats. At later ages, high abundance was maintained for tPA but collagenase decreased substantially between ages PN21 to PN35. The location of collagenase was found to be associated with bone-lining cells, osteoblasts, osteocytes and in the matrix along cement lines. In contrast, tPA was associated with endothelial cells lining the blood vessels entering bone. The presence and developmental expresion of these two proteinases appeared to be unaffected by the exposure to zero gravity. The calvarial thickness of the pups was also examined, again the exposure to zero gravity showed little to no effect on the growth of the calvariae. Notably, from G20 to PN14, calvarial thickness increased dramtically, reaching a plateau after this age. It was apparent that elevated collagenase expression correlated with rapid bone growth in the period from G20 to PN14. To conclude, collagenase and tPA are present during the development of rat calvariae. Despite being produced by the same cell *in vitro*, the osteoblast, they are located in distinctly different places in bone *in vivo*. Their presence, developmental expression and quantity do not seem to be affected by a brief exposure to zero gravity *in utero*.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Margolis, R.N., Canalis, E., and Partridge, N.C. Anabolic hormones in bone: Basic research and therapeutic potential. J. Clin. Endo. Metab., 81, 872-877 (1996).

Partridge, N.C. and Winchester, S.K. "Osteoblast proteinases" in "Principles of Bone Biology." Edited by: Bilizekian, J.P., Raisz, L.G., and Rodan, G.A. Academic Press, San Diego, CA. pp 207-216, 1996.

Partridge, N.C., Walling, H.W., Bloch, S.R., Omura, T.H., Chan, P.T., Pearman, A.T., and Chou, W.-Y. The regulation and regulatory role of collagenase in bone. Critical Reviews in Eukaryotic Gene Expression, 6, 15-27 (1996).

Pearman, A.T., Chou, W.-Y., Bergman, K.D., Pulumati, M., and Partridge, N.C. Parathyroid hormone induces c-*fos* promoter activity in osteoblastic cells through phosphorylated CREB binding to the major CRE. J. Biol. Chem., 271, 25715-25721 (1996).

Microgravity and Placental Development

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No Co-Is Assigned to this Task

Funding:

Project Identification: 5-01131
Initial Funding Date: 1/96
FY 1996 Funding: $72,330
Joint Agency Participation: NIH

Solicitation: 93-OLMSA-03
Expiration: 12/96
Students Funded Under Research: 0

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)
Responsible NASA Center: ARC
Flight Hardware Required: Animal Enclosure Module

Task Description:

This experiment will use pregnant rats to determine the effect of microgravity in development of the rat placenta. Ten pregnant rats will be aboard the space shuttle during its 11-day mission. Upon return to Earth, the rat uteruses and placentas will be examined. Morphological, biochemical, and endocrine variables of these tissues will be analyzed to determine whether the cells involved 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.

Due to technical difficulties related to the acquisition and preparation of glucose transmitter cDNA probes, little progress has been made with this the remaining specific aim of the project during 1996. However, we have recently completed preparation of RNA samples and blotting in preparation for Northern analysis of glucose transmitters. Reliable probes have been identified and we will be completing this aspect of the project within the current budget year (ending 4-30-97). Once these data are completed, a manuscript will be submitted to Biology of Reproduction a leading journal in the field of reproductive biology

Expression of two placental hormones (placental lactogen I and II) which are linked to placental growth and differentiation was not influenced by space flight.

Infertility is a health problem which may lead to significant psychological and economic stress for many couples. This malady may result from processes associated with gamete fertilization, embryo implantation, or placental insufficiency. This project was designed to study the latter of these possibilities by examining the role of gravity in placental growth and development. It was hypothesized that the correct vectoral movement of cells during embryo implantation and placental development is dependent upon gravitational forces. In addition hemodynamic changes associated with microgravity were postulated to adversely influence placental development and function. There is a need to know the effect of space flight on reproductive processes at this time to determine if studies of mammalian biology which would require stable animal colonies aboard an orbiting

laboratory can be planned. Also, a better understanding of reproductive processes will facilitate management of human proliferation in face of a finite supply of resources on Earth.

Differentiation and Tropisms in Space-grown Moss (*Ceratodon*)

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Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $79,996

Joint Agency Participation: NSAU

Solicitation:

Expiration: 9/96

Students Funded Under Research: 4

Flight Information:

Flight Assignment: CUE (STS-87, 11/97)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC-LED

Task Description:

Protonemata of the moss *Ceratodon* are tip-growing cells that grow up in the dark. This cell type is unique compared to cells in almost any other organism since the growth of the plant cell itself is completely oriented by gravity. Thus, both the processes of gravity sensing and the gravity response occur in the same cell. Gravity sensing appears to rely upon amyloplasts (starch-filled plastids) that sediment. This sedimentation occurs in specific zones and plastid zonation is very complex with respect to plastid morphology, distribution, and gravity. Microtubules may function both in producing gravitropic curvature as well as in controlling plastid sedimentation. Since gravity plays crucial roles in the growth and organization of this highly specialized cell, it is important to determine whether this cell differentiates normally in microgravity. Protonemata are excellent subjects for spaceflight experiments since they are readily cultured in sealed containers in the dark. Thus they can be grown in a BRIC and fixed in position in space.

Ceratodon protonemata are also an excellent system for the study of gravity on the organization since differentiation is influenced by light. Thus, it is possible to study the effects of microgravity on several different cell types. Since these cells are phototropic (grow towards unilateral light) as well as gravitropic, space flight offers an opportunity to resolve whether both phototropism and gravitropism occur simultaneously.

In many plants, starch content is influenced by gravitational stress and by microgravity. Since *Ceratodon* protonemata contain different types of amyloplasts (statolith and storage), it will also be meaningful to test the effects of flight on starch content and metabolism.

A key component of this mission is the involvement of Ukrainians. All Ukrainian scientists collaborating on this moss experiment have been working for many years in both ground- and space-based studies in moss gravitational biology.

During the FY96, the culture and experiment conditions for the two moss species, *Ceratodon purpureus* (US part of the experiment) and *Pottia intermedia* (Ukrainian part), have been optimized. Different moss strains were tested, the size of the inoculum defined, and culture conditions like temperature, the substrate components, the pH of the substrate, the duration of incubation were optimized. Precautions to avoid floating of the biological material during chemical fixation were developed. The light quality, light intensity, and the experiment treatments for the space flight were defined and finalized.

Furthermore, experiment-specific hardware has been designed and constructed for the SPM experiments during the FY96. BRIC canisters have been modified and reconstructed to provide illumination for the moss samples during flight utilizing red light emitting diodes and fixation of these samples at the end of the experiment treatment. For the first time it will be possible to chemically fix biological samples in place (petri dish) while growing in μg. This useful hardware will find numerous applications for future space flight experiments.

In ground-based reference experiments, the influence of gravity versus light was analyzed. The negative gravitropism as well as the positive phototropism of protonemal tip cells were documented and analyzed in detail. It became obvious that in higher light intensity conditions, gravitropism is overwritten by the phototropic reaction. To determine whether gravitropism is entirely inhibited by unilateral red light, the direction of the light and gravity vectors relative to one another were varied and the effects of different light intensities were tested. Dark-grown negatively gravitropic cultures were illuminated with red light (660 nm) at intensities less than or equal to 1 Mol s-1 m-2. After 24 h, 85% of all protonemata were oriented directly towards the light. To detect any integration of both stimuli, comparable cultures were turned at various angles with respect to gravity and then illuminated so that the light and gravity vectors acted either in the same or different directions. The mean angle and variability of the phototropic reaction were comparable regardless of position with respect to gravity. At higher light intensities, growth is oriented strictly by phototropism, not gravitropism. Intensities between 0.1 and 1 μMol result in more variable growth angles, but net phototropism is still obvious. Below 0.1 μMol, phototropism is no longer present and gravitropism is detectable statistically, although the protonemata are not uniformly up-right. These results are consistent with the hypothesis that red light brighter than a 0.1 μMol threshold switches off gravitropism. This is supported by analysis of an aphototropic mutant which is gravitropic in red light except when phytrochrome function is restored by exogenous biliverdin. As a result of these experiments, one low light treatment of 50 nMol s-1 m-2 has been implemented into the finalized flight experiment schedule.

Clinostat (gravity compensation), centrifuge (hyper-G) and experiments utilizing aphototropic mutants of *Ceratodon* are in preparation. Furthermore the focus in upcoming reference experiments will be to analyze the organelle distribution in dark-grown versus red light-grown moss protonemata under 1-G and clinostat conditions and to determine the utilization of the Steedman's wax embedding procedure for the analysis of these questions.

Moss protonemata grow solely by tip growth, that is the most apical cell of the filament extends only at its tip. Dark-grown protonemata of mosses such as *Ceratodon*, *Physcomitrella*, *Funaria*, and *Pottia* grow upward in the dark and are this negatively gravitropic. Gravitropic, tip-growing cells of plants are unique compared to cells in almost any other organism, since the growth of the plant cell itself is completely oriented to gravity. Protonemata of the moss genus *Ceratodon* are among the most vigorous and rapid gravitropic tip-growing cells known. Gravity sensing seems to take place continuously in *Cera-adon* rotonemata. The apical tip cell contains amyloplasts that sediment in a specific zone just behind the apex and several lines of experimental evidence suggest that this sedimentation functions in gravity sensing. These cells offer many experimental advantages for studying the effects of gravity since they: (1) represent one of the few gravitropic tip-growing systems known, (2) contain both the processes of gravity sensing and the gravity response in the same cell, (3) are also phototropic and light influences gravitropism, and (4) are readily cultured in agar in sealed containers in the dark. Although space flight experiments have been conducted with moss protonemata (*Funaria hygrometrica* on board the Russian orbital space station Salyut 6, 1979), none involved gravitropic cells. *Ceratodon* protonemata are an excellent system in which to study the interactions of phototropism with gravitropism and these experiment features will reveal a new understanding of these basic biological processes.

FY96 Publications, Presentations, and Other Accomplishments:

Kern, V.D. and Sack, F.D. Gravitropism vs. phototropism in protonemata of the moss *Ceratodon.* 5th Bi-annual Conference on Gravitational Biology, Colby-Sawyer College North, New London, NH, July 14-19, 1996.

Kern, V.D. and Sack, F.D. (abstract) Gravitropism and phototropism in *Ceratodon purpureus.* ASGSB Bull., 10 (1), 54 (1996).

Sack, F.D., Schwuchow, J., and Kern, V.D. (abstact) Gravitropism in high density media supports intracellular, statolith-based sensing in moss protonemata. ASGSB Biology Bull., 10 (1), 54 (1996).

Wagner, T.A., Cove, D.J., and Sack, F.D. "A positively gravitropic mutant mirrows the wild-type protonemal response in the moss *Ceratodon*" in "Plants in Space Biology," ed., Suge, H. Institute of Genetic Ecology, Tohoku University, Japan, 53-60 (1996).

Microgravity Effects during Fertilization, Cell Division, Development, and Calcium Metabolism in Sea Urchins

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Co-Investigators:

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Funding:

Project Identification: A144 BF78 A48 9700
Initial Funding Date: 10/95
FY 1996 Funding: $97,077

Solicitation: 95-OLMSA-01
Expiration: 9/96
Students Funded Under Research: 3

Flight Information:

Flight Assignment: ARF-1 (STS-77, 5/96)
Responsible NASA Center: KSC
Flight Hardware Required: ARF

Task Description:

Gravity has been shown to affect bone calcium, and it may well influence processes during fertilization, cell division, development, and embryogenesis. This project explores the role of microgravity during fertilization, early development, cytoskeletal organization, and skeletal calcium deposition in a model developmental system: the sea urchin. In doing so, we have also helped the Canadian Space Agency (CSA) develop, test, and fly the aquatic research facility (ARF) system. During FY96, this system was tested successfully at the Kennedy Space Center and we demonstrated 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. In addition, 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).

Following the ground-based studies, sea urchins were flown in the ARF system aboard the space shuttle Endeavor during the STS-77 mission in May 1996. In-flight fertilization of six cultures was successfully accomplished by astronaut Mario Runco using the newly designed Fertilization Syringe Unit (FSU). Six other unfertilized egg cultures were fertilized in a 1-G centrifuge in space to mimic gravity conditions on Earth and to serve as control to the cultures grown in 0-G.

The methods developed for this project have been applied to one-cell, two-cell, eight-cell, and sixteen-cell embryos, as well as to blastulae, gastrulae, and pluteus stages grown in the chambers specifically designed for space shuttle experimentation. We have used fluorescently labeled anti-tubulin antibodies to detect microtubules, fluorescently labeled anti-centrosomal antibodies to detect the microtubule-organizing centers, and the fluorescent compound Hoechst 22358 to visualize chromosomes. We have also performed high resolution electron microscopy to detail the molecular components of cytoskeletal structures at 0g and 1g.

Activities for the past year started with the completion of a Science Verification Test to verify that the Aquatic Research Facility (ARF) and the Fertilization Syringe Unit (FSU) could support (a) in flight fertilization, (b) development of sea urchin embryos up to the pluteus stage, and (c) in-flight fixation. Specifically:

1. The compatibility of all hardware components with gamete viability was tested and the materials were found to be non-toxic, and semi-permeable to O_2 & CO_2.

2. Maintenance of in-flight parameters including temperature, G-force, timing of sperm addition, and fixative addition were verified.

3. Gamete ratios and volumes of sperm to eggs and sperm/eggs to seawater within the specimen enclosures were determined.

4. Appropriate fixatives, concentrations, and volumes were studied with respect to seawater volumes and total volume constraints of the ARF specimen enclosures and fixation subsystem.

The highlight of this year's accomplishment was the successful launch and completion of our experiments on the Space Shuttle Endeavor from May 19 - May 26, 1996. In-flight fertilization was accomplished by astronaut Mario Runco, who used the FSU for the first time in flight. Unfertilized egg cultures were also fertilized in a 1-G centrifuge in space to mimic gravity conditions on Earth and to serve as control to the cultures grown in 0-G.

Monoclonal and polyclonal antibodies against cytoskeletal components were utilized in combination with confocal immunofluorescence microscopy and molecular electron microscopy to characterize the behavior of microfilaments, microtubules and their organizing centers, and the centrosomes, under space flight conditions. Structural and molecular analysis revealed that fertilization took place in space but that the sperm-induced calcium release of cortical granule contents was incomplete. Secretion was altered and membrane fusion of cortical granules with the plasma membrane was delayed and irregular. The fertilization-associated microvillar elongation was reduced and development was slower when compared with ground controls. The underlying molecular basis for the reduction of microvilli formation was the reduced polymerization of actin into elongated microfilaments. This finding correlates well with findings by others on tissue culture cells that had been flown in space. It is also supported by other studies in our group on insect culture cells of *Drosophila* in which microfilament polymerization was altered when cells were cultured on clinostats.

Other findings revealed that 4% of all dividing cells exhibited abnormal division patterns. We interpret this finding to be the result of improper function of the centrosome-centriole complex, which showed abnormal behavior. This may indicate that the centrosome-centriole complex is gravity-sensitive. We now would like to build on these data and investigate the underlying molecular mechanisms of this phenomenon.

As of today, the function of centrioles remains obscure and it was hypothesized that they may be organelles sensing the direction for cell movement. On an evolution model, centrioles may have been key organelles during development to adapt to a gravity environment. When adaptation was completed, these organelles may have lost their function. This hypothesis will be interesting to test in microgravity.

Aside from being inclusions in centrosomes, 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 gastrula 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 in the KC-135, it was observed that swimming of sperm is slowed down as compared to control sperm.

Several aspects of this research are aimed at understanding diseases that affect humans on Earth. Since the cytoskeleton is important for many processes in the cell including signal transduction, hormone secretion,

organelle transport, cell shape changes, fertilization, cell division, and cell polarity,these studies will provide an important foundation for studies on osteoporosis, neurological diseases, aging, and reproduction. Data from the studies may help in identifying target sites for pharmaceutica which would interfere with pathological conditions found in osteoporosis and other diseases related to the cytoskeleton.

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 also include processes which relate to 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 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 our knowledge of diseases of the muscle, bone, and nerve systems 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 affects 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 ionic and calcium requirements for specific events in cells and tissue. Secretion is a major event during numerous 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.

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.
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 the 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. Cilia are often 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 systems. It has been shown 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 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 is 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 muscles. 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 the ground prove that the ARF system can now be utilized to perform experiments in space requiring controlled temperature, humidity, and 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 employed 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.

FY96 Publications, Presentations, and Other Accomplishments:

Chakrabarti, A., Lavin, C., and Schatten, H. (abstract) The centrosome-centriole complex during sea urchin development: Light and electron microscopy analysis. Molecular Biology of the Cell, December Abstracts, (1996).

Schatten, H. and Chakrabarti, A. (abstract) Culture of sea urchin embryos in the new Aquatic Research Facility (ARF), and first results from its maiden space voyage on STS-77. ASGSB Bulletin, 10 (1), (October 1996).

Brain-Pituitary Axis Development in the Cebas Minimodule

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Funding:

Project Identification:

Initial Funding Date: 7/96

FY 1996 Funding: $100,000

Solicitation: 95-OLMSA-01

Expiration: 6/99

Students Funded Under Research: 11

Flight Information:

Flight Assignment: CEBAS (STS-89, 1/98)

Responsible NASA Center: ARC

Task Description:

The CEBAS minimodule system is a man-made aquatic ecological system that incorporates animals, plants, snails, and microorganisms. It has been proposed that the CEBAS will lead to a multigeneration experimental facility for utilization in a space station as well as for the devlopment of an aquatic CAELSS to produce animal and plant biomass for human nutrition. In this context, research on the reproductive biology of the organisms within the system should receive the highest priority. Thus, the goals of our proposal are to provide information on space-flight-induced changes in the brain-pituitary axis and in the organs that receive information from the environment in the vertebrate selected for the CEBAS Minimodule program, the freshwater teleost *Xiphophorus helleri* (the swordtail). We will study the development of the brain-pituitary axis in embryos, neonates, immature and mature swordtails using histology, cytology, immunohistochemistry, morphometry and *in situ* histochemistry to evaluate the synthesis, storage, and release of neurotransmitters, neuroregulatory peptides, neurohormones, and pituitary hormones as well as the structure of the organs and cells that produce, store, or are the target organs for these substances. Similar methods will be used to study the pineal organ and the olfactory system.

This research represents a logical sequence of studies, from laboratory to space flight, that will provide seminal, essential information of the effect of space travel conditions on the development and functioning of the neuroendocrine system regulating the reproductive system.

I. Tissue specimens

The vertebrate animal model that will inhabit the CEBAS minimodule is the freshwater teleost, *Xiphorphorus helleri,* commonly known as the swordtail. In September 1996, tissue samples from adult and immature swordtails were received from Bochum, Germany. A small number of these samples were already sectioned and mounted on glass slides. Most were whole brains and bodies, in alcohol. Whole tissue specimens were sectioned and mounted at Brooklyn College during FY96.

II. Immunocytochemistry

Antisera listed below, needed for immunocytochemical mapping of neuropeptides, were analyzed for optimum working dilutions and for cross reactivity with molecularly similar antigens. Specificity of the antisera was determined by absorption reactions. These studies were conducted using sections of adult and immature swordtails, of both genders, that we had received from Bochum.

a) rabbit anit-(porcine) Neuropeptide Y (Peninsula Co.); optimum staining dilution was found to be 1:200 with ABC Elite process

b) rabbit anit-(porcine) dynorphin (Peninsula Co.); optimum staining dilution was found to be 1:200 with ABC Elite process

c) rabbit anit-(human) androgen receptor (Affinity Bioreagents); optimum staining dilution was found to be 1:300 with ABC Elite process

d) rabbit anit-(synthetic) neurotensin (Chemicon); optimum staining dilution was found to be 1:400 with ABC Elite process

e) rabbit anit-(porcine) galanin (Chemicon); optimum staining dilution was found to be 1:400 with ABC Elite process

f) rabbit anit-(synthetic) FMRF-amide (Incstar); optimum staining dilution was found to be 1:400 with ABC Elite process

III. Testing of Tampa Bay water

In order to standardize conditions and eliminate variables that might interfere with the proper analysis of our future data, effects of life in Tampa Bay water is being evaluated. In keeping with this goal, 75 gallons of Tampa Bay water was received and divided among three 20-gallon tanks. Three other tanks containing conditioned Brooklyn water were set up at the same time. Neonatal siblings, born at Brooklyn College but derived from Bochum stocks, were divided equally among the six tanks. These neonates will be weighed, measured, and staged for sexual development every ten days until they reach sexual maturity.

The implications of studying a vertebrate in space flight with a reproductive system similar to mammals is patent. Additionally, knowledge of the reproductive system of fish may serve to introduce the consideration of aquaculture as a means of generating animals protein into life support systems.

FY96 Publications, Presentations, and Other Accomplishments:

Blum, V., Andriske, M., Eichhorn, M., Kreuzberg, K., and Schreibman, M.P. A controlled aquatic ecological life support system (CAELSS) for combined production of fish and higher plant biomass suitable for integration into a lunar or planetary base. Acta Astronautica, 37, 361-371 (1995).

Blum, V., Andriske, M., Kreuzberg, K., and Schreibman, M.P. "A novel approach to combined animal and plant biomass production for human nutrition in closed-loop systems. Advances in modern biotechnology" in "New Opportunities in Animals, Plants, Industry and Social Development." Edited by: Estrada, M.P., et al. Proc. Biotechnologia Habana '95, 3, pp 1-10, 1995.

Blum, V., Andriske, M., Kreuzberg, K., and Schreibman, M.P. Animal protein production modules in biological life support systems: Novel combined aquaculture techniques based on the Closed Equilibrated Biological Aquatic System (C.E.B.A.S.). Acta Astronautica, 36, 615-623 (1995).

Blum, V., Andriske, M., Kreuzberg, K., and Schreibman, M.P. The closed equilibrated biological aquatic system as a tool for the development of novel aquaculture systems. Adv. Space Res., (in press).

Blum, V., Andriske, M., Voeste, D., Behrens, H., Kreuzberg, K., and Schreibman, M.P. The C.E.B.A.S. Mini Module: Design of the spaceflight hardware, scientific experiments and future aspects for bioregenerative life support system research. Preprint 46th International Astronautical Congress, October 2-6, 1995, Oslo, Norway.

Breuckmann, A., Paris, F., Schreibman, M.P., and Blum, V. Immunoreactive gonadotropin-releasing-hormone (GnRH) in the brain and pituitary of adult and juvenile swordtails (*Xiphophorus helleri, Teleostei, Poeciliidae*). J. Morphol., 230, 55-67 (1996).

Schreibman, M.P. and Magliulo-Cepriano, L. Brain-pituitary axis development in *Xiphophorus*: A progress report. Proceedings of the 12th CEBAS workshop. R. Braucker, editor, Ruhr University, Bochum, Germany, p. 47-49, 1996.

Schreibman, M.P. and Magliulo-Cepriano, L. Recent advances in brain-pituitary axis development in *Xiphophorus*. Proceedings of the 11th CEBAS workshop. R. Braucker, ed., Ruhr University, Bochum, Germany, 1995, p. 173-176.

Effects of Microgravity on Microbial Physiology

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Funding:

Project Identification: E562
Initial Funding Date: 02/96
FY 1996 Funding: $187,800

Solicitation: 93-OLMSA-07
Expiration: 09/98
Students Funded Under Research:

Flight Information:

Flight Assignment: STS-95 (Target)
Responsible NASA Center: JSC
Flight Hardware Required: AMS (Automated Microbiology System)

Task Description:

Microorganisms are capable of quickly adapting to changes in their environment. Metabolic pathways adjust to new environmental conditions to optimize growth and ensure survival. Environmental adaptation is a key tool used in microbial research to investigate the basic physiology of microbial species. Temperature, pH, and growth media constituent manipulation are classic examples of environmental parameter control schemes used to induce observable metabolic changes. While classic induced physiology adaptation has been studied extensively, the effects of gravity on microbial physiology remain essentially unknown. Prior space flight research indicates that exposure to microgravity can lead to increased growth rates and increased resistance to antimicrobial agents. However, the limited data and conflicting results of this body of work demand that a well-controlled, prolific methodology be adapted for microbial space flight research. The hypothesis to be tested here is that microgravity will induce functional changes in microbial physiology. To identify and evaluate these changes, a two-phase project will be conducted to first screen for and then target statistically significant differences in physiological behavior between ground-based and on-orbit microbial cultures. The screening phase of the project will investigate the growth, substrate utilization characteristics, and reaction to antimicrobial agents of twelve microbial strains including bacteria and yeasts. These microbes will be analyzed simultaneously on-orbit and on the ground using an automated microbial analysis technology, the Vitek System manufactured by bioMerieux Vitek, Inc. Microbial growth patterns will be measured in standard Vitek identification and antibiotic susceptibility test cards. A physiological profile consisting of observed growth rates, substrate utilization characteristics, and antimicrobial susceptibilities will be obtained for each microbial strain. Physiological profiles from the terrestrial and on-orbit microbial analyses will be compared to determine statistically significant differences in behavior. In the second phases of the project, Vitek test cards containing unique media formulations and/or antimicrobial agents will be developed to further investigate each of the specific metabolic differences identified in the screening phase. These test cards will be analyzed in subsequent shuttle flights to elucidate the cellular mechanisms responsible for the observed metabolic anomalies. This study will provide a basic understanding of the effects of microbiology on microbial physiology. In addition, the antimicrobial

physiological profile data will aid in the development of effective therapeutic treatment for controlling on-orbit infectious disease. Adaptation of the Vitek instrument for space flight will provide the first ever on-orbit comprehensive microbial identification capability, an invaluable capability for clinical and environmental microbiology on future long-duration space missions.

The Microbial Physiology Flight Experiment (MPFE) project was initiated in earnest in February of 1996. Progress through the end of fiscal year 1996 fell into two primary categories: hardware development and experiment science refinement. A prototype Automated Microbiology System (AMS) reader/incubator instrument had been previously designed and fabricated under a McDonnell Douglas Aerospace (MDA) Internal Research and Development (IRAD) project. Functional testing of this unit was completed in January, 1996. While designed for a shuttle middeck locker configuration, the prototype AMS unit required materials upgrades and internal structural design modifications to prepare for flight-certification testing. After these upgrades were performed, the AMS instrument was sent to NASA JSC for engineering evaluation testing. A series of tests including bench shock, thermal cycling, random vibration, acoustic noise and electromagnetic compatibility were performed to assess the maturity and integrity of the basic design. All test results were at or near middeck requirement levels. The only recorded failure was loss of power after vibration testing in the second of three axes—a failure attributed to insufficiently anchored internal power cables. The instrument was returned to MDA for final processing in preparation for verification and certification testing of the unit for use as a flight back-up. Project science activities focused on refinement of specific flight protocols and selection of microorganism/Vitek analytical card combinations for the first phase of the MPFE experiment. Of primary interest from the perspective of on-orbit operations was the issue of containment of the microbial strains. A contamination control plan was developed in preparation for presentation to the Payload Safety Review Panel. A prioritized list of microorganisms was developed by the microbial strains. A contamination control plan was developed in preparation for presentation to the Payload Safety Review Panel. A prioritized list of microorganisms was developed by the project co-investigators, and these strains were matched with appropriate Vitek identification and susceptibility test cards. Susceptibility cards were selected so as to maximize the number of anti-microbial agents which elicited intermediate resistance from each selected microbial strain. This will provide for the ability to measure either an increase or decrease in susceptibility when exposed to microgravity. A Strain Stability Test supporting study test plan was then developed to determine the effect of refrigerated stowage on the growth of the selected strains. Since the microbes will be inoculated into their respective Vitek test cards on ground and then stowed at 4°C until the time of analysis in the AMS instrument, it is necessary to determine how long each strain remains stable in this refrigerated condition. Strains which loose characteristic growth and susceptibility patterns quickly will be scheduled for analysis early in the flight, while stable strains will be scheduled for later in the flight.

The Microbial Physiology Flight experiment (MPFE) is aimed at identifying gravity-dependent physiological processes by comparing the growth and metabolism of a broad range of microorganisms cultured in space and on the ground. The anticipated results will indicate which microbes and specifically which metabolic pathways are affected by variation in the gravitational environment. These results will benefit the fields of biology, clinical microbiology, and applied microbiology.

From a basic biological research prospective, identification of gravity-dependent physiological processes will add significantly to our overall understanding of how the environment, and gravity in particular, affects the basic processes of life. Bacteria and yeast are some of the simplest forms of life, yet they exhibit physiological pathways that have been conserved through millions of years of evolution. In this way, microbes represent the ideal model for studying the most basic life processes. The simplicity of these organisms allows for targeting of specific bioprocesses without the complications of intercellular and system-level interactions. Gravity represents an under-utilized tool in the array of environmental parameters used to elicit metabolic responses. Gravity is unique in this array in that it is the one environmental constant under which all forms of life evolved. For this reason, it will be especially intriguing to observe how microbes adapt to an environmental parameter which their genetic programming has never encountered. One might suggest that observed microbial adaptive behaviors may be extrapolated to help explain observed adaptive physiological phenomena in humans as well.

Clinical microbiology is a field which may benefit significantly from the MPFE project. Prior work has indicated that microbes exhibit an increase in resistance to antimicrobial agents when grown in microgravity. If this behavior can be confirmed, the flight experiment will provide an excellent opportunity to investigate in detail the mechanisms of antibiotic resistance. The growing prevalence of resistant pathogen strains has made antimicrobial research of critical importance to the health of the public as a whole. Results of the MPFE project will provide a unique set of data for the clinical microbiology community to use in combating infectious disease.

Gravity-dependent bioprocesses are of considerable interest in the field of applied microbiology as well. Biochemists and crystallographers currently take advantage of the minimal gravity on the Shuttle in Earth orbit to produce crystals that would otherwise not be possible. Physicists researching combustion and phase-change phenomena utilize the unique conditions of microgravity to investigate processes that cannot occur here on Earth. What about biological processes? Biological gravitational effects are not so easily anticipated as those for physical phenomena, yet it is these effects which one hopes will make space processing a valuable commodity. This project will constitute the first steps in the process of identifying those microgravity-induced bioprocesses which may be harnessed to produce unique biochemicals and pharmaceuticals in space.

Effect of Spaceflight on Development of Immune Responses

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Funding:

Project Identification:

Initial Funding Date: 5/94

FY 1996 Funding: $

Solicitation: 93-OLMSA-03

Expiration: 4/96

Students Funded Under Research: 1

Flight Information:

Flight Assignment: NIH-R1 (STS-66, 11/94)

Responsible NASA Center: ARC

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.

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. Analysis of the data were completed in this grant period. Analysis of the results of this study suggest that alterations in leukocyte subset distribution similarly occur in fetuses and pups. Additional analysis of the data is continuing. Cytokine production of dams was reduced after flight, as expected. Cytokine production of pups showed a trend toward reduction after flight, but differences were not statistically significant. The results of this study indicate the some 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 and the supporting grant were ended and terminated in FY96.

This study has been designed to determine the effects of space flight on development of immune responses in offspring of flown pregnant rats. It should provide new information regarding the normal development of the immune response. This information could prove useful in enhancing the understanding of the development of the immune response in humans. Such understanding could provide new information that may be potentially applicable to understanding the mechanism of and treatment human childhood immunological disorders.

FY96 Publications, Presentations, and Other Accomplishments:

Sonnenfeld, G., Miller, E.S., Jr., Mattei, M., Morton, D., Bailliard, F., Fowler, N.A., Swiggett, J.P., Hakenwirth, A.M., Bates, R., and Morris, V. Spaceflight and development of immune responses. American Society for Gravitational and Space Biology, October, 1995.

Role of Thyroxine in Space-Developed Jellyfish

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Funding:

Project Identification:
Initial Funding Date: 10/95
FY 1996 Funding: $115,500

Solicitation: 93-OLMSA-02
Expiration: 9/96
Students Funded Under Research: 3

Flight Information:

Flight Assignment: ARF-2 (TBD)
Responsible NASA Center: KSC
Flight Hardware Required: ARF-2

Task Description:

The metamorphosis process which enables the formation of ephyrae from polyps is influenced by a hormone, jellyfish thyroxine (JF-T4), which is synthesized following iodine administration. Two groups of polyps in space (but not controls) formed ephyrae without iodine administration. In addition, in space, jellyfish ephyrae lost most statoliths and swam/pulsed abnormally. These findings suggest that JF-T4 synthesis, utilization, or secretion may be different in space as compared with ground controls.

Our tasks this year were focused on preparing for the ARF-2 jellyfish-in-space experiment. Our efforts included growing and testing numerous jellyfish for normality, including the testing of polyps and ephyrae in an SCA (Sample Container Assembly) of the ARF (Aquatic Research Facility) provided by the Canadian Space Agency. In addition, methods for the fixation of jellyfish for ultrastructural studies and for the measurement of JF-T4 were improved.

Testing: Prior to receipt of an SCA, we tested the rate of segmentation of polyps and the development and release of ephyrae in tissue culture flasks following iodine treatment at 22°C. We compared results from these tests with animals tested at 28°C. Polyps form their first segment within 3 days at 28°C and within 5 days at 22°C. Free-swimming ephyrae are found after 6 days at 28°C and after 10 days at 22°C. We also tested the asexual progeny of polyps which were used for the IML-2 experiment by inducing ephyra formation. Of 43 ephyrae which developed in seven tests, >80% had 8 arms, were good swimmers and had normal morphology. It is important to use animals which have a high fidelity for the normal 8-arm number to clearly establish whether microgravity will affect this feature during the ARF-2 experiment as it apparently did during the IML-2 experiment.

After receipt of an SCA in July, we tested polyps in 30 ml of artificial sea water (ASW) in the SCU (Sample Container Unit) of the SCA at 22°C to determine how many polyps would metamorphose in this small amount of fluid. Six tests were done between July and October 1st, five of them using 10 polyps in 30 ml iodine in

ASW per test to induce ephyra formation. Ephryae developed in all of the SCUs although some required 14 days to complete development at this temperature. Most of the ephyrae had normal numbers of arms (8) and normal morphology. However, many of the ephyrae did not swim. In a different test, 100 polyps in 30 ml ASW were induced to metamorphose in the SCU with iodine on Day 4. All of the polyps remained normal and began segmenting three days after they were given iodine. The results from all of these tests were very encouraging as to the biocompatibility of the SCAs and led to subsequent tests with higher numbers of animals and a slightly higher temperature.

Jellyfish Cultures: Cultures of polyps were grown in our laboratory this year in preparation for the ARF-2 flight. Polyps are fed brine shrimp larvae once weekly and transferred to clean ASW in clean dishes with a pipet. As of September 1996, we had 200 hand care cultures totalling approximately 20,000 polyps. Of these, 4000 polyps are from the same group of polyps that gave rise to ephyrae with predominantly 8 arms during the IML-2 experiment. In addition, seven aquaria containing approximately 7,000 polyps each are fed brine shrimp once weekly and their ASW is filtered. We are also developing new cultures by sub-culturing ten polyps from cultures which have been shown to give rise to predominantly 8-armed ephyrae. These cultures will also be used for the ARF-2 experiment if they give rise to predominantly 8-armed ephyrae. In addition to our existing cultures, we started a new culture of polyps from a medusa collected from nature last summer. These animals will be tested to determine arm numbers when sufficient animals are available.

Fixation for Ultrastructural Studies: For our fixation studies, we simulated flight conditions for 10 ephyrae in tissue culture flasks containing 30 ml of ASW and 1 ml of 70% glutaraldehyde. One group of ephyrae was exposed to the glutaraldehyde for 1 day and another group for 2 days simulating fixation at 24h and 48h before shuttle return. These groups were post-fixed in glutaraldehyde for 1.5h and stored in buffer for at least 24h before exposure to osmium for 1h. Examination of ultrathin sections from the graviceptors and striated muscles of some of these ephyrae revealed them to be suitably fixed for TEM studies. Likewise ephyrae graviceptors examined with the SEM were determined to be well-fixed. Two similar groups prepared as those mentioned above were stored in ASW with sucrose instead of buffer. This procedure was done to determine whether flight ephyrae and controls in the ASW/sucrose solution during transfer to Eastern Virginia Medical School from KSC post-flight were acceptable after post-osmication. Ephyrae from the group prepared in glutaraldehyde for 2 days and post-fixed and stored in ASW for 24h were found to be well-fixed for both SEM and TEM ultrastructural studies.

SEM/TEM of Hair Cells and Muscle: We tested procedure modifications for the SEM and TEM since we plan to do ultrastructural studies of muscle and the touch-plate area of graviceptors following the ARF-2 flight. For the SEM studies, we explored the use of a lower pH during processing to remove glycocalyx material which may obscure hair bundles of hair cells. For TEM studies, we found that the addition of tannic acid following fixation with glutaraldehyde and post-fixation with osmium results in cells with better membrane preservation and staining. We will use these preparative procedures with the ARF-2 fixed ephyrae.

Jellyfish Thyroxine (JF-T4) Studies: JF-T4 hormone levels were measured using 60 polyps induced to strobilate with 10-5M I125 in ASW. HPLC measurements taken at 48h and 72h post-iodination reveal the 72h JF-T4 level is several fold greater than at 48h. We are evaluating several JF-T4 hormone preservation methods (including 20mM NaOH in 80% ethanol) which appear to stabilize the hormone for a considerable period of time. JF-T4 in ASW is being concentrated using C18 syringe columns to prepare the samples for T4 RIA analyses.

This experiment is designed to provide a new understanding of the basic biological processes involved in the utilization of thyroid hormones(s) for development of biological organisms on Earth as well as in space. Thus far, little is known about the effects of thyroid hormone(s) on developing higher organisms in space partly because the time required for completion of mammalian development far exceeds the current time periods of shuttle flights. Tiny jellyfish polyps, however, synthesize a thyroid-type hormone which induces the development of jellyfish of new form, ephyrae, within a week. These organisms provide a rapidly developing model system for the quantitation of the hormone and its receptors(s) in space (and ground controls) during its

synthesis and utilization. Through this flight experiment, we will be able to investigate the role that this hormone plays in: (a) the differentiation of new structures such as graviceptors with statoliths and hair cells; (b) the differentiation of a new neuromuscular (motor) system; and (c) demineralization of statoliths. The information gained from these studies will help us to understand the role that the hormone and its receptor(s) play on Earth in the differentiation of similar structure in mammals, including humans. 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-thyroxine, may be synthesized in greater amounts in space. If so, then thyroid hormones of mammals may be synthesized in higher amounts, possibly giving rise to a hyperthyroid condition in mammals, particularly those maintained in space for long time periods. If such a hyperthyroid condition were to occur in pregnant mammals in space for long periods, the increased hormone production could impact fetal development. Further, a knowledge of specific detrimental microgravity effects on hormone production and function could lead to the development of countermeasures to prevent such effects in animals (including humans) in space. The jellyfish research, therefore, could ultimately contribute information needed to achieve human long-term occupancy in microgravity on the space station or during long-term space travel.

In addition to the increased understanding of the effects of the thyroid-type hormone in space, a comparison of microgravity effects with ground controls could lead to a better understanding of the role that gravity plays in developing animals on Earth, especially regarding their thyroid hormone and receptor synthesis, distribution, and/or utilization.

FY96 Publications, Presentations, and Other Accomplishments:

Spangenberg, D.B. The effects of weightlessness on *Aurelia* budding and ephyra development: Final report. Investigator's Working Group meeting, European Space Agency, Frascoti, Italy.

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Spangenberg, D.B., Lattanzio, F., Philput, C., Schwarte, R., Lowe, B., and Philput, J. (abstract) Effects of weightlessness on *Aurelia* budding and ephyra development. ASGSB Bulletin, 9, 88 (1995).

Effects of Microgravity on Tobacco Hornworm (Manduca Sexta) *During Metamorphosis*

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 89-13 OSSA IML-2

Initial Funding Date: 10/95

Expiration: 9/96

FY 1996 Funding: $63,861

Students Funded Under Research: 0

Flight Information:

Flight Assignment: BRIC-04 (STS-70, 6/95) and BRIC-07 (STS-77, 6/96)

Responsible NASA Center: KSC

Flight Hardware Required: BRIC

Task Description:

Studies on altered orientation of tobacco hornworms (*Manduca sexta*) pupa relative to gravitational field have shown changes of some amino acids, rate of adult development, and flight muscles. All of these parameters are dependent on ecdysone levels which are elevated by reorienting the insect into a head-up vertical position. The following studies were undertaken to examine the effects of microgravity on tobacco hornworm ecdysone release and subsequent development. Pupae were loaded into passively controlled biological research canisters (BRICs), placed in a shuttle mid-deck locker, and recovered 9 days after launch. Examinations revealed suspended development in both flight and 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 was carried out in space flight using modified BRIC hardware to study the development of the gravity sensitive development of tobacco hornworm. The insects successfully developed during the BRIC-07 experiment (STS-77). Data indicate that space flight caused development to be slowed by about 15%.

BRIC-07 was successfully flown and analyses of the samples were to be completed during the remainder of calendar year 1996. Thus to the end of FY96, we had shown that tobacco hornworms could be successfully flown in space as evidenced by significant development. The system to protect the insects during the stresses of launch and landing worked flawlessly. The slower development during flight likely reflects an effect of weightlessness on the insect's endocrine system. However, the duration of the flight was too long to obtain meaningful endocrine data.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Webster-McElvogue, K., Bayomi, S., Ochoa, M., O'Connor, D., Peterson, E., Polanco, R., and Tischler, M.E. (abstract) Use of BRIC hardware to study adult development of the tobacco hornworm (*Manduca sexta*). ASGSB Bull., 10, 48 (1996).

Effect of Spaceflight on TGF-β Expression by hFOB Cells

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Funding:

Project Identification:

Solicitation: 93-OLMSA-04

Initial Funding Date: 1/95

Expiration: 12/95

FY 1996 Funding: $

Students Funded Under Research: 2

Flight Information:

Flight Assignment: NIH-C4 (STS-69, 7/95) and NIH-C6 (STS-80, 1996)

Responsible NASA Center: ARC

Task Description:

Weightlessness results in skeletal wasting in astronauts. The bone loss is similar to that which occurs in people who undergo prolonged bedrest or, in some cases, lose the use of one of their limbs due to injury or disease. The exact cause of the bone loss is not yet clear but is at least partially due to decreased activity of osteoblasts, the cells which produce the matrix which mineralizes to become bone. Weightlessness results in decreased bone formation in rodents as well as humans. Studies performed on rats implicate a protein which is produced by bone cells and is important in the communication between cells. The gene for for that protein was found to be expressed in bone at reduced levels following space flight, but that level was dramatically increased (within 24 hours) when normal activity was reestablished following space flight.

This experiment which was flown on STS-639 and STS-80 was designed to determine whether gene expression is reduced in cultured bone cells following space flight and if so how quickly the levels return to normal after flight. Results from this experiment will help us determine the usefulness of cultured bone cells in understanding how the acceleration due to gravity functions to maintain bone cell activity.

The cells to be used in this study are unique. They have been altered to allow them to grow nearly indefinitely at a low temperature (35°C), but when cultured at a higher temperature (39°C), they stop growing and become mature osteoblasts which synthesize bone matrix. This experiment will study the effects of weightlessness and recovery on the mature form of the osteoblast-like cells.

Our efforts in FY95 and FY96 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-ß expression on cultured osteoblast-like cells, 2) performing a realistic dry run at the Kennedy Space 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, both flight experiments were a success. The results show that hFOB cells grow during space flight in a similar manner to on Earth. Furthermore, the media was sampled at selected intervals

during the NIH-C6 flight and analyzed for accumulated type 1 collagen and prostaglandin E2. Following space flight, total cellular RNA was isolated from the flight and ground control cells. Steady-state mRNA levels were determined by Northern blot analyses and RNase protection assays for growth factors (TGF-ß1 and TGF-ß2), bone matrix proteins (type 1 collagen and osteocalcin), and genes unlikely to be altered by space flight (GAP and L32). The results demonstrate that spaceflight has little or no effect on the genes surveyed in cultured bone cells. We are currently analyzing other genes as well as the histological characteristics of the cells.

The long-term objectives of this research are to understand the cellular and molecular mechanisms which mediate skeletal adaptation to mechanical usage. Weight bearing is essential to establish and maintain the normal balance between bone formation and bone resorption that functions to achieve and preserve bone volume. Skeletal unweighting, whether due to space flight, prolonged bedrest, paralysis, localized stress shielding following arthroplasty, or cast immobilization leads to bone loss and an increased risk for fractures. We hypothesize that cyclical mechanical stimulation has direct effects on osteoblasts to modulate expression of one or more signaling peptides (growth factors). In turn, these osteoblast-derived regulatory peptides may act on osteoblasts to regulate bone matrix synthesis, osteoclasts to regulate bone resorption, and on osteoblast and osteoclast progenitors to regulate the proliferation and subsequent differentiation of these cells to osteoblasts and osteoclasts. An exciting aspect of this model is that it identifies a rational means of intervention to prevent disuse osteopenia; it should be possible to mimic the protective effects of weight bearing in the unloaded skeleton by regulating the local levels of the appropriate bone cell derived signaling peptides. The focus of these studies is the TGF-ß, an important osteoblast-derived skeletal growth factor whose expression is regulated by weight bearing. We have shown that mRNA levels for TGF-ß 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-ß 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.

FY96 Publications, Presentations, and Other Accomplishments:

Dobnig, H. and Turner, R.T. (poster) Programed intermittent sc infusion of parathyroid hormone (PTH) in sexually mature rats: Effects on bone and mineral metabolism. 10th Intl. Congress of Endocrinology, San Francisco, CA, June 1996.

Dobnig, H., Zhang, M., and Turner, R.T. (poster) Parathyroid hormone (PTH) induced modulation of bone lining cells to osteoblasts is preceded by changes in steady-state mRNA levels for proto-oncogenes and skeletal growth factors. 10th Intl. Congress of Endocrinology, San Francisco, CA, June 1996.

Evans, G.L., Bryant, H.U., Magee, D.E., and Turner, R.T. Raloxifene inhibits bone turnover and prevents further cancellous bone loss in adult ovariectomized rats with established osteopenia. Endocrinology, 137, 4139-4144 (1996).

Evans, G.L., Morey-Holton, E., and Turner, R.T. (poster) Spaceflight has compartment specific effects on mRNA levels for bone matrix proteins and bone matrix production in rat femur. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Firling, C.E., Wakley, G.K., Evans, G.L., Sibonga, J., and Turner, R.T. Lack of an effect of sodium Zeolite A on rat tibia histomorphometry. J. Bone Miner. Res., 11, 254 -263 (1996).

Harris, S.A., Evans, G.L., Kidder, L.S., Spelsberg, T.C., and Turner, R.T. (poster) Effects of spaceflight on human fetal osteoblastic cell gene expression and physiology. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Harris, S.A., Tau, K.R., Turner, R.T., and Spelsberg, T.C. "Estrogens and progestins" in "Principles of Bone Biology." Edited by: Belizikian, J.P., Raisz, L.G., and Rodan, G. Academic Press, New York, pp 507-520, 1996.

Kidder, L.S., Schmidt, I.U., Evans, G.L., and Turner, R.T. (poster) Estrogen (E) as well as growth hormone (GH) is essential to prevent cancellous osteopenia in hypophysectomized (HYPOX) rats. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Oursler, M.J., Kassem, M., Turner, R., Riggs, B.L., and Spelsberg, T.C. "Regulation of bone cell function by gonadal steroids" in "Ostereoporosis." Edited by: Marcus, Feldman, Kelsey. Academic Press, (in press).

Sibonga, J.D., Bell, N.H., and Turner, R.T. (poster) Ibuprofen reduces cancellous bone of the tibia and blocks the effects of tamoxifen to prevent bone loss in response to ovariectomy in growing female rats. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Sibonga, J.D., Evans, G.L., Hauck, E.R., Bell, N.H., and Turner, R.T. Ovarian status influences the skeletal effects of tamoxifen in adult rats. Breast Cancer Res. Treat., 41, 71-79 (1996).

Turner, R.T. "The skeletal response to arthroplasty: Role of growth factors in bone turnover" in "Joint Replacement Arthroplasty, 2nd Edition." Edited by: Morrey, B.F. Churchill Livingtston, New York, Ch. 10, pp 95-106, 1996.

Turner, R.T. "Organ selective actions of tamoxifen and other partial antiestrogens" in "Organ-Selective Actions of Steroid Hormones" (eds. Baird, D.T., Shatz, G., and Krattenmacher, R.). Ernst Schering Research Foundation Workshop 16, Springer Verlag, Heidelberg, Germany, pp 65-84, 1995.

Turner, R.T. and Dobnig, H. (poster) The origin of osteoblasts during bone remodeling. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Vidovszky, T.J., Bronk, J.T., Witkiewicz, H., Meyer, W.A., Turner, R.T., Ilstrup, D.M., An, K.N., Rock, M.G., Morrey, B.F., and Bolander, M.E. (poster) Accelerated production of UHMW-PE debris in the sheep: A possible model for bone loss around femoral stem. Orthopaedic Research Society, Orlando, FL, February 1996.

Vidovszky, T.J., Witkiewicz, H., Turner, R.T., Rock, M.G., Morrey, B.F., and Bolander, M.E. Expression of metalloproteinases and TIMPs in periprosthetic tissues of failed total hip arthroplasties. Orthopaedic Research Society, Orlando, FL, February 1996.

Westerlind, K.C., Morey-Holton, E., Evans, G.L., Tanner, S.J., and Turner, R,T. (poster) TGF-beta may help couple mechanical strain and bone cell activity in vivo. American Society for Bone and Mineral Research, Seattle, WA, September 1996.

Effect of Space Travel on Skeletal Myofibers

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No Co-Is Assigned to this Task

Funding:

Project Identification:

Solicitation: 93 OLMSA-04

Initial Funding Date:

Expiration:

FY 1996 Funding: $

Students Funded Under Research: 7

Joint Agency Participation: NIH, WRAIR

Flight Information:

Flight Assignment: NIH-C2 (STS-66, 11/94) and NIH-C5 (STS-72, 1995)

Responsible NASA Center: ARC

Task Description:

This experiment will use tissue-cultured muscle cells to study the effects of space flight on muscle atrophy, protein turnover rates, and growth factor secretion to determine whether tissue-cultured skeletal muscle fibers exposed to microgravity will atrophy in the same way as fibers in humans and other animals. The lack of tension on muscles in space due to the lack of gravitational force, offers the opportunity to study the cellular mechanisms that cause microgravity-induced atrophy.

This type of research may help identify and develop countermeasures required if people are to sustain muscle strength on long-duration space voyages. The experiment will also provide a rapid screening system for testing drugs to prevent muscle atrophy.

The 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 (November 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 countrols. 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 (STS-72) 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.

FY96 Publications, Presentations, and Other Accomplishments:

Vandenburgh, H.H. Keystone Symposium on Tissue Eng., Taos, NM, 1996.

Vandenburgh, H.H. Center for Eng. in Medicine, Mass. Gen. Hosp., Boston, MA, 1996.

Vandenburgh, H.H. Marine Biological Laboratory, Woods Hole, MA, 1996.

Vandenburgh, H.H. Am. Soc. Mech. Eng., Atlanta, GA, 1996.

Vandenburgh, H.H., Chromiak, J.A., Shansky, J., and Del Tatto, M. (abstract) Effects of space travel on cell metabolism and protein turnover of skeletal muscle cells. Am. Soc. Cell Biol., Special Session, H59, 1996.

Vandenburgh, H.H., Chromiak, J., Shansky, J., and Del Tatto, M. (abstract) Initial International Space Station (ISS) definition studies for examining the effects of long term space travel on tissue cultured mammalian skeletal myofibers. ASGSB Bull., 10, 19 (1996).

Vandenburgh, H.H., Del Tatto, M., Shansky, J., LeMaire, J., Chang, A., Payumo, F., Lee, P., Goodyear, A., and Raven, L. Tissue engineered skeletal muscle organoids for reversible gene therapy. In Vitro Cell Dev. Biol., 32, 53A (1996).

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Functional Development in a Model Vestibular System

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification:

Initial Funding Date: 10/95

FY 1996 Funding: $162,277

Solicitation: 93-OLMSA-07

Expiration: 9/96

Students Funded Under Research: 3

Flight Information:

Flight Assignment: ARF-3 (TBD)

Responsible NASA Center: KSC

Flight Hardware Required: ARF

Task Description:

Development of the gravity-sensing organs in altered gravity conditions has been studied in several series of experiments, with variable and often conflicting results reported. On IML-2 (STS-65) we flew pre-fertilized eggs of the Japanese red-bellied newt, *Cynops pyrrhogaster*, staged such that they would not produce any otoconia (the dense "stones" in the inner ear, upon which gravity exerts its force) before they reached μ-G. Newt eggs offer a favorable preparation in which to study vestibular-system development since their inner ear progresses from very primitive stages to a nearly adult form in the 16-day time available for a space shuttle flight. Our original hypothesis was that growth of the otoliths would be affected by the G field, with larger otoliths being formed in μ-G partially compensating for the decreased weight of a given mass in μ-G. In the week after shuttle landing, the utricular and saccular otoliths were of nearly identical volume in the newt larvae reared in μ-G and in the ground-control larvae. However, amphibians possess a second system of otoconia, produced in the endolymphatic sac (ES). The ES communicates with the saccule via the endolymphatic duct (ED). In the flight-reared newts, the volume of otoconia in the ES was 3 to 4 times larger than in ground-control animals at the same developmental stage. At later stages, when the otoconia from the ES move through the ED to the saccule, these additional stones cause the saccular otolith to be larger in the flight-reared larvae. We used a Japanese X-ray micro-focus system to follow growth of the otoliths in one flight-reared larva for 9 months after return. The saccular otolith volume was significantly larger in this animal, compared to several ground-control animals from the same batch of eggs, for 5 months post-flight, with the largest difference seen at 2 months post-flight. This had behavioral consequences in that the flight-reared larva exhibited abnormal head position, with the head raised 30° from the horizontal for its entire terrestrial life (normal newts keep their head horizontal).

In experiments performed in Japan using parabolic flight, we identified stereotypic behavioral responses to transient exposure to μ-G at different developmental stages which correlated with the development of different portions of the vestibular system. Using the Canadian Aquatic Research Facility (ARF) we will rear additional newt larvae in small (35ml capacity) aquaria. One set will be reared at μ-G by having the centrifuge rotate at

only one revolution per ten seconds (to equalize environmental conditions among the six arms of the centrifuge), and the other set will be reared at 1-G by having the centrifuge run continuously at 80 RPM. Video facilities are available to observe behavioral responses to the first exposure to μg, produced by briefly stopping the 1-G centrifuge and observing the response to the first exposure to 1-G, produced by rotating the quasi-static centrifuge at 80 RPM for several minutes late in the flight.

Measurements of the otolith-ocular reflex were made in flight- and ground-reared larvae from IML-2. Although the techniques used at that time were not fully adequate, the results indicate that the gain of the reflex increases with developmental stage in ground-reared larvae, as reported by others. In the flight-reared larvae, there was considerable variability in the measured gain, but there was no indication of a systematic change in gain with development. This indicates that the reflex, by which the animal uses gravitational stimulation of the otoliths to stabilize an image on the retina, does not develop normally in the absence of gravity. Improved measurement techniques are being developed for use on ARF-3.

In summary, the main aspects of the IML-2 experiment will be replicated using a facility that allows in-flight 1-G controls. Approximately half of the larvae will be maintained for several months after flight in an attempt to verify the indication that the greatest changes in both the endolymphatic and saccular otoconia occurred after the larvae were introduced to 1-G conditions.

In the ARF system, specimens are kept in 35 ml aquaria (two aquaria on each of six arms on the two centrifuges). Since we do not plan to fix specimens in space, our samples can be maintained with double containment but this still requires any oxygen the larvae consume to be either contained in the water before loading or to diffuse through the gas-permeable membranes in the walls of the aquaria. Thus, we need to confirm that newt larvae will survive and develop normally for periods comparable to flight duration in the aquaria. We have had inseminated newts, captured and maintained in hibernation, shipped from Japan to San Antonio and some of these animals have been brought out of hibernation, injected with hormone (Human Chorionic Gonadotropin) and then laid viable eggs. In several runs, both aquaria in one Sample Container Unit (SCU) were loaded with 10 fertilized eggs (approximately developmental stage 20) and they survived for 30 to 60 days. Thus, it appears that there will be no problem in keeping specimens alive in the SCUs. We are currently comparing developmental rates between larvae maintained in the SCUs with that of larvae from the same adults maintained in open containers with better aeration.

With our enhanced interest in the otoconia formed in the endolymphatic sac, we have undertaken more detailed developmental studies of the ES and ED. The first otoconia appearing in the ES have been found in small crypts in the wall of the sac. These crypts have been reconstructed from serial sections, which demonstrates that the crypts empty into the ES and ED. It appears that the cells forming the crypts pump enough Ca^{++} into the lumen of the crypt to precipitate the aragonitic otoconia typical of the endolymphatic system. Two manuscripts describing this development and otoconial formation have been sent to colleagues for comment before submission for publication.

A resident in the Neurosurgery program at our medical school has asked to spend a year in our laboratory and will work on improving our methods for measuring the otolith-ocular reflex. Larvae will be restrained and rotated about their long body axis and counter-rotation of the eyes will be observed. We will consult with investigators at JSC who have developed a sophisticated computer image-analysis system to measure human ocular torsion. If we can adapt features of their system to improve measurement of ocular counter-rotation in the newt, we will be able to make more reliable measurements of the otolith-ocular reflex and test for differences in the development of the reflex between animals reared with gravity acting on the otolith and those without gravitational pull on the otoliths, in the larvae reared on quasi-static centrifuge in flight.

It is well known that animals and man lose calcium from their bones during extended periods in space. Our studies are designed to help understand what processes control biomineralization. There is growing evidence that the lack of gravity can adversely affect bone mineralization even in isolated embryonic bones. Thus, there appears to be a fundamental interaction between mineralization and gravitational forces. Such an interaction

could have major consequences in a developing gravity-sensing organ which depends on the 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. Since normal development on Earth always occurs in a 1-G field, the effect of that gravity has largely been neglected. If the otolith-ocular reflex were to develop abnormally in μ-G, this could imply abnormal development if animals were maintained in an altered position during critical developmental stages on Earth.

There is evidence that the otoconia in elderly humans can become decalcified, which would cause a decreased mass with which to sense gravitational and linear-acceleration forces. This has been suggested to contribute to unsteadiness in elderly humans. Our studies of the mechanisms by which otoconia are mineralized will help to clarify the processes of demineralization as well.

Another pathological condition in humans, Benign Paroxysmal Positional Vertigo (BPPV) occurs when otoconia, usually restricted to the utricle and saccule, become attached to the cupula overlying the sensory hair cells in the semicircular canals which sense angular acceleration. The cupula is normally neutrally buoyant in the endolymph, but when it becomes loaded with dense otoconia, it will "sink," giving the patient the sensation that he is spinning, as would be the case during normal stimulation of the semicircular canal. The prevailing hypothesis is that the "extra" otoconia on the cupula have become dislodged from the saccular or utricular otoliths. However, if those otoconia were to be formed by some abnormal chemical condition in the endolymph, similar to the formation of otoconia in the ES crypts in the newt, this would suggest a more specific etiology for BPPV which might be treatable medicinally. We have been in contact with several neuro-otological surgeons who perform surgery to block the semicircular canals in BPPV subjects. If we could obtain some otoconia from such subjects, we can test their crystallography, using Fourier Transform InfraRed (FTIR) spectroscopy. The otoconia in the utricle and saccule are made of calcium carbonate in the calcite crystal form, whereas those formed in the ES in the newt are $CaCO_3$ in the aragonite crystal form. If otoconia in the semicircular canals of BPPV patients were to be composed of aragonite, this would strongly suggest that they are not displaced otolith otoconia and could be formed by a mechanism similar to that found in the newt.

FY96 Publications, Presentations, and Other Accomplishments:

Pedrozo, H.A., Weiderhold, M.L., Schwartz, Z., and Boyan, B.D. (abstract) Mechanism of statoconia production and homeostasis under normal and hypergravity conditions. ASGSB Bull., 9 (1), 40 (1995).

Pedrozo, H.A., Schwartz, Z., Harrison, J., Weiderhold, M.L., Dean, D.D., and Boyan., B.D. Evidence for the involvement of carbonic anhydrase and urease in calcium carbonate formation in the gravity-sensing organ of *Aplysia californica*. Oral Presentation. Eighth Annual Meeting and Workshop of the Texas Mineralized Tissue Society, Columbus, Texas, May 10-12, 1996.

Pedrozo, H.A., Schwartz, Z., Luther, M., Dean, D.D., Boyan, B.D., and Weiderhold, M.L. Mechanisms of adaptation to hypergravity in the statocyst of *Aplysia californica*. Hearing Res., 102, 51-62 (1996).

Steyger, P.S. and Weiderhold, M.L. Visualization of newt aragonitic otoconial matrices using transmission electron microscopy. Hearing Res., 92 (1/2), 184-191 (1996).

Weiderhold, M.L. The pond snail statocyst: A new model gravity-sensor for space research. Department of Otolaryngology-Head and Neck Surgery Alumni Day Lecture. Video Presentation. The University of Texas Health Science Center, San Antonio, Texas, June 22, 1996.

Weiderhold, M.L., Gilpatric, K.D., and Hejl, R.J. (abstract) Otolith systems in newt larvae reared in microgravity on IML-2. ASGSB Bull., 9 (1), 88 (1995).

Weiderhold, M.L., Gilpatric, K.D., Hejl, R.J., Koike, H., and Nakamura, K. (abstract) Development of otoliths and endolymphatic otoconia in newt larvae reared in microgravity. Abstracts of the Nineteenth Midwinter Research Meeting of the Association for Research in Otolaryngology, 19, 145 (1996).

Weiderhold, M.L., Gilpatric, K.D., Hejl, R.J., Koike, H., and Nakamura, K. Development of Otoliths and Endolymphatic Otoconia in Newt Larvae Reared in Microgravity. Oral Presenation. Nineteenth Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, Florida, February 4-8, 1996.

Compact, Rapid Response Optical Air Quality Monitor

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Funding:

Project Identification: 199-04-17-19
Initial Funding Date: 6/96
FY 1996 Funding: $100,994

Solicitation: 95-OMSA-01
Expiration: 9/99
Students Funded Under Research:

Task Description:

The proposed program will develop and demonstrate a compact, rapid response air quality monitor for multiple gasesous contaminants. This monitor meets an identified need in advanced technology development for enclosed human environments in space. The sensor technology is based on miniature, room-temperature diode laser absorption in the near-IR between 1.3 and 3.3μm. Capability already under development at Physical Sciences Inc. (PSI) for CO, CO_2, NH_3, NO_2, NO, and HF detection will be augmented to include sensitive detection of volatile hydro/halocarbon compounds such as: acetone, benzene, cyclohexane, freons, ethanol, propanol, chlorodifluoromethane, dichlorobenze, and ethyl acetate. The volatile organic compounds (VOCs) will be detected using vibration overtone/combination band absorption near 1.75 μm. In the first year, a broadly tunable external cavity diode laser will be used to complete spectral surveys of target VOCs to quantify absorption strengths and potential interferences in order to identify optimum spectral regions for selection of monolithic, fiber-coupled diode lasers. In the second and third years, a prototype flight version will be developed and tested. This prototype will use mutiple, fiber-coupled lasers to detect multiple species in a compact, light-weight, low-power consumption electro-optic package with no moving parts. AlliedSignal will work with PSI as a subcontractor to assist in the prototype development and the preliminary design of a flight package.

In the first three quarters of the first year of the program, progress has focussed on FTIR measurements of the fundamental absorption strengths of selected volatile organic compounds recommended by the NASA Environmental Monitoring Requirements Document. For the first time, quantitative measurements of overtone and combination band absorption between 1.5 and 2.0 μm have been determined for the following compounds: acetone, benzene, methanol, toluene, 1,2-dichloroethane, and 1,1,1 tri-chloroethane. Room-temperature diode laser measurements of CO, HCN, and HCl with detection limits on the order of 1 ppm were also demonstrated. PSI also entered into a Technology Cooperation Agreement with NASA researchers at the Jet Propulsion Laboratory to incorporate emerging near-room temperature diode lasers in the 1.8 - 3.0 μm spectral region into our tests. These new lasers are potentially important for the present program because they access stronger bands of many target molecules and should improve detection limits. Delivery of the first of these lasers for testing is expected during the last quarter of this year.

The progress during the past year has included numerous detection "firsts" regarding an important class of compounds in space-borne and terrestrial industrial habitats. Many of the compounds on the NASA Environmental Monitoring Requirements Document list include common industrial solvents whose concentration are regulated in work environments. PSI envisions commercial markets for the technology

developed as a part of this program and has already begun commercialization of several individual gas sensors. The volatile organic compounds are also representative of unburned hydrocarbons from fossil fuel combustion and we expect additional applications in this market as well.

Microbial Monitoring Based on Quantitative PCR

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Funding:

Project Identification: 199-04-17-22

Initial Funding Date: 5/96

FY 1996 Funding: $220,152

Solicitation: 95-OLMSA-01

Expiration: 4/98

Students Funded Under Research:

Task Description:

The monitoring of spacecraft life support systems for the presence of health threatening microorganisms is paramount for crew well being and successful completion of missions. Currently most environmental samples are assayed using conventional microbiology techniques that require skilled technicians and elaborate culture media, and sometimes days before obtaining results.

The union of the molecular biology techniques of DNA probe hybridization and polymerase chain reaction (PCR) offers a powerful method for the detection, identification, and quantification of microorganisms. This technology is theoretically capable of assaying samples in as little as two hours with specificity and sensitivity unmatched by any other method. This probe-hybridization/PCR has recently come of age in a technology called TaqManTM, invented by Perkin Elmer. Instrumentation using TaqMan concepts is evolving towards devices that can meet NASA's needs of size, low power use, and simplicity of operation. The chemistry and molecular biology needed to utilize these probe-hybridization/PCR instruments must evolve in parallel.

Our project will establish the chemical and molecular biological tools necessary to use the emerging TaqMan technology for monitoring environmental microbes. In a collaboration between the University of Alabama at Birmingham, Perkin Elmer, and NASA's Marshall Space Flight Center (MSFC), we will develop methods using both commercially available as well as Perkin Elmer's next generation Taqman instrumentation, and then test the new methods on water recycled by the MSFC water reclamation system. We will create sets of PCR primers and TaqMan probes that can specifically detect bacteria, fungi, protozoa, and viruses belonging to a list of microbial species and groups we believe could be detrimental to a spacecraft environment. We will establish optimal biochemical methods for sample preparation that would be amenable to the kind of fully automated instrumentation space utilization will require, as well as detailed PCR protocols for quantitative microbial detection.

In addition to space utilization, a microbial monitor will have tremendous terrestrial applications. Analysis of patient samples for microbial pathogens, testing industrial effluent for biofouling bacteria, and detection of biological warfare agents on the battlefield are but a few of the diverse potential uses for this technology.

An Advanced Approach to Simultaneous Monitoring of Multiple Bacteria in Space

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Funding:

Project Identification: 199-04-17-10
Initial Funding Date: 3/94
FY 1996 Funding: $308,705

Solicitation: 93-OLMSA-07
Expiration: 3/97
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 achieved in the chemistry, microbiology, and engineering components of the program were synergistically demonstrated in the second year through the detection and quantification of a mixture of microorganisms (*E. Coli* and *V. proteolyticus*). First, based on discussions with the NASA microbiology group, six key organisms were chosen for the detection targets for testing the first generation instrumentation and methods. Subsequently algorithms were developed to identify the probes which were extremely specific to key organisms while possessing at least three mismatched bases to all other organisms in the Ribosomal Database. Three probes in the 13 to 19 nucleotide length were identified as being capable of detecting 2,227 of the 2,347 species of bacteria.

In addition to selecting the optimal set of probes for the DNA microarrays, probe/target hybridization selectivity was significantly enhanced. A naturally occurring peptide (SAL-A1) was employed to selectively drive duplex formation in the absence of buffer salts, confirmed by exhaustive kinetic binding analyses. Also, to counter the association rate limitations caused by the inherent 16S target strand secondary structure, a new "sandwich" assay

was developed which utilizes a "chaperone probe" that serves to open the 16S molecule in the region near the binding site for hybridization to the surface bound probe.

Engineering progress included the refinement of the proximal CCD detector/imager which successfully detected and imaged 970 zeptomoles of labeled DNA. Moreover, a microarray printer was developed capable of efficiently dispensing 96 element DNA probe microarrays within a $1cm^2$ area.

Collectively all components were synergistically demonstrated by the quantitative detection of a mixture of *E. Coli* and *V. proteolyticus* microorganisms hybridized to a DNA probe microarray fabricated by the prototype microarray printer and imaged by the refined proximal CCD detector/imager.

The primary objective of the microbial analyzer is to provide a miniaturized, automated microbial analysis system capable of rapidly monitoring air and water supplies, as well as identifying particular pathogens in mission environment. The research would have a far reaching effect on monitoring the environment for manned missions to Mars and other planets in the 21st century from the orbiting space station. The highly sensitive proximal CCD detection procedure would also provide an ideal platform to support automated, low cost DNA sequence analysis for diagnostic applications on Earth. Moreover, the microbial analyzer would be very suitable for routine monitoring for water treatment facilities and hospitals due to its high sensitivity and miniature format.

FY96 Publications, Presentations, and Other Accomplishments:

Balch, B., Eggers, M., Gangadharan, R., Hogan, M., Mallik, A., McMahon, M., and Mendoza, L. Quantitative detection of molecules utilizing chemiluminescent and fluorescent reporter groups. Clinical Chemistry, September (1996).

Eggers, M. A biochip for rapid molecular detection. IBC Conference on Diagnostics for Gene Detection of Infections Agents and Human Genetic Diseases, San Diego, CA (May 2-3, 1996).

Eggers, M. Geosensor technology. Third international conference on automation in mapping and DNA sequencing, Berkeley, CA (November 5-8, 1995).

Eggers, M., Hogan, M., Mallik, A., Powdrill, T., McMahon, M., Gangadharan, R., Balch, B., Yang, H., Jamieson, N., and Lamture, J. A versatile biochip for gene-based diagnostics. Proceedings of Electro '96, Somerset, NJ (April 30 - May 2, 1996).

Mallik, A., Powdrill, T., Eggers M., and Hogan, M. Matrix analysis of biochip array hybridization. IBC Biochip Array Technologies Conference, San Jose, CA (March 18-19, 1996).

Advancement in Determining Hazardous Volatile Organic Compounds in Air

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-04-17-13
Solicitation: 93-OLMSA-07
Initial Funding Date: 3/95
Expiration: 3/98
FY 1996 Funding: $145,148
Students Funded Under Research: 1

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.

The research program as originally conceived contained four major themes and these encompass the overall goal of advancing the understanding and practice of ion mobility spectrometry. The main four categories are: explorations in the origins or foundations of ion mobility spectra, creation of a comprehensive data base of IMS spectra for volatile organic compounds, creation of artificial intelligence to automatically identify IMS spectra, and creation of Windows driven IMS software. A final category involved secondary issues of hardware design such as non-radioactive sources and extended linear ranges in response with IMS drift tubes. Progress on each of these categories with a summary of relevant activity is described in the individual sections below.

Foundations for Ion Mobility Spectra

During FY96, essential issues in the creation of ion mobility spectra were delineated and a preliminary model for the formation of spectra was proposed and tested in limited manner. Experimental studies with the rates of decomposition of gas phase proton bound dimers in air at sub-ambient temperatures were used to validate the model of the origins of IMS spectra and to reveal the nature of ion instabilities in the ion drift region of an IMS drift tube. These models and studies (now in draft form for submission to journals by June 1997) have been the first such contributions in the twenty-seven year history of IMS and constitute a major milestone in delineating the origins of ion mobility spectra (an original goal of the proposed research program). These accomplishments are due to the presence in my laboratory of Prof. J.A. Stone (Queens University, Ontario who was a sabbatical fellow on this project in my laboratory) and of Dr. R.G. Ewing (who, under NASA funding on this project,

completed his Ph.D. in October 1996). Present understandings have caused us to explore the stability of ions at elevated temperatures, necessitating the creation of a high temperature equivalent to the specialized drift tubes used heretofore in these studies. This instrumentation has been crafted in FY96 and studies are planned for July 1997.

In summary, we have for the first time, a model for the origins of ion mobility spectra and an understanding of IMS response toward organic chemicals. This constitutes the completion at a satisfactory stage one of a major objective of the research program (naturally, we wish to extend these studies and are presently doing so). While we expected that this milestone would be completed in the last year of this project (FY97), success came in FY96. This has significant consequences for the balance of research to be completed and will dramatically advance our capabilities in the second category, described in the next section.

Expansive Data Base

Another goal for FY96 was the assembly of GC-IMS hardware and the creation of an expansive data base of IMS spectra for ca. 180 compounds at several conditions of temperature and moisture. The essential preliminary aspects of this part of the project were completed in FY96 including the establishment of chromatographic conditions, formulation of the composition of mixtures, the control of moisture in the drift gas, and the assembly of hardware including a high temperature drift tube mated to a gas chromatograph. Moisture in drift gases now can be controlled from 100 ppb to 10,000 ppm and temperature of the drift tube can be controlled from 25°C to 300°C. Data handling protocols are nearly completed and actual data collection should commence in June 1997. This start date is a delay from our original schedule and is associated with challenges in the creation of a high temperature drift tube for the GC-IMS hardware. Nonetheless, the critical aspects of control of parameters has been confirmed and tested over a period of several months for baseline control.

In summary, all the tools necessary to rapidly complete this section of work are in place and the data base will be completed in summer 1997. An existing data base has been used to feed into a near parallel part of our research program, namely, artificial intelligence for IMS spectra through neural networks and other methods.

Neural Network Studies

Our team commenced with examination of full spectra by neural networks using upgraded software (NeuralWare for Windows) mid-year in FY96 and efforts are underway presently in the evaluation of what preprocessing is necessary for success with neural networks. Software and methods have been under daily operation and explorations are continuing with specialized personnel. We have invited and received Dr. Suzanne Bell (Eastern Washington University) into our program as an external consultant on this and have also placed a preliminary data base with Dr. A.P. Snyder at the US Army for exploration. Thus, our philosophy has been to use multiple paths for exploration of neural network processing. At present, results suggest that the use of whole spectra are influenced by slight variations or variance in drift times and normalization of drift times and ion intensities are necessary. In summary, the AI tools are in place and in use and studies are progressing. Eventually, the expansive data base will be entered into these activities.

Integrated Windows Software

During FY96, an attempt was made successfully to create a Windows-driven software package for IMS signal processing. This package was created using TestPoint for Windows and the results, while functional, were regarded as only partially satisfactory. An assessment is underway to determine a satisfactory path to pursue in FY97. In FY96, a small IMS company (PhemtoScan) released a beta-test version of Windows driven software and we are in discussions to team with this group and attach automated identifications into their software. This subject has taken secondary importance to the previous issues.

Other Issues in Instrumentation

Two areas of alternate ion sources and extended linear ranges were identified in the proposed research plan though neither of these issues is especially relevant to the use of GC-IMS on space station. Rather, the subjects have major relevance in technology transfer from NASA to environmental monitoring applications. An early examination on extended linear ranges in response were first explored in FY95 at University of Manchester

Institute of Science and Technology (UMIST). This work has continued at UMIST with by personnel trained by the PI. However, no experimental efforts were devoted to this at NMSU in FY96. Instead, efforts were continued at UMIST and findings from this work will be incorporated into NMSU efforts when warranted. Efforts on non-radioactive sources were negligible in FY96 so resources could be devoted to the two top categories above and efforts are underway to initiate studies of a beta-version non-radioactive source from a commercial concern for FY97.

OVERALL ASSESSMENT OF PROGRESS IN FY96

We have arrived at models for the origin of IMS spectra a year in advance of when we thought this might actually arise. We are prepared in all ways to commence data collection on an expansive data base. No problems are anticipated in completion of this during summer 1997 and we will be only a few months behind the proposed schedule in this category. We are on schedule with neural network processing of spectra. The success in our first category is clearly the most important scientific accomplishment in this project for FY96 and FY95.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Ewing, R.G. (dissertation) Kinetics decomposition of proton bound dimer ions with substituted amines in ion mobility spectrometry. Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM, (Oct. 1996).

Plasma Chemical Approaches to the Development of Biofilm-Resistant Surfaces

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 199-04-12-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 3/95
Expiration: 3/98
FY 1996 Funding: $145,200
Students Funded Under Research: 0

Responsible NASA Center: ARC

Task Description:

This research is concerned with applying the techniques of low-temperature plasma polymerization and plasma surface modification of polymers to the development of antibacterial or biofilm-resistant coatings or surfaces on plastics, metals, or other substrata that could be used in a variety of aerospace, biomedical, or commercial applications. For NASA's Advanced Life Support programs, a major application would be conferring lasting biofilm resistance upon the piping or container walls in the closed-loop water reclamation system (WRS) for future space habitats. Biofilms on the surfaces of the WRS could harbor potentially pathogenic bacteria in the recycled water coming from showering, clothes laundering, or dishwashing and thereby present a hazard to astronauts on long-duration space missions as in the future International Space Station. Other applications, which could emerge as NASA technology transfer to the commercial sector, are antibacterial coatings in domestic or industrial systems involving humid environments (e.g., air conditioning units) or in the biomedical field (e.g., urinary catheters and intravascular devices). Since biofilms, once established, resist physical cleaning and penetration by biocides, their formation must be avoided or suppressed. This research aims to follow up unpublished results at Ames Research Center (ARC) which indicated that a coating (designated here as PPOM, and prepared by the plasma polymerization of a certain organic monomer, OM), imparted biofilm resistance to polyethylene, glass and other substrata when exposed to a pure *Pseudomonas aeruginosa* culture. Accordingly, this research has as a major goal the study of the biofilm resistance of a family of coatings derived from the plasma polymerization of various organic monomers structurally related to OM when those coatings are exposed not only to *P. aeruginosa* but to other common pathogenic bacteria as well. The working hypothesis is that there is a particular chemical functionality (or functionalities) within the complex PPOM structure responsible for the observed antibacterial effect, and this notion needs to be tested by determining the relative biofilm resistance of a series of PPOM-like coatings containing varying amounts of the putative functionalities. At the same time, there is much scientific interest in determining the relative biofilm resistance of a homologous series of commercial plastics that are the conventional polymer analogues of the PPOM-like plasma polymers. Studies of both classes of polymers should lead to important structure-property relationships, something that has been lacking in the considerable literature on bacterial attachment to assorted polymers.

Research during the second year of this three-year NRA grant continued to be concerned with plasma chemical approaches to biofilm-resistant coatings or surfaces. As before, the work involved the joint efforts of a plasma chemist and a polymer chemist at Ames Research Center (ARC) to produce and characterize a series of

plasma-deposited polymeric coatings, and the subsequent extramural assessment of the biofilm resistance of those coatings when exposed to pure *Pseudomonas aeruginosa* culture. However, as stated in the FY95 Task Progress report, a "second opinion" on the reproducibility of early ARC results was needed as a follow-up to disappointing biofilm test results obtained during the first year by a microbiologist at Southwest Texas State University. Consequently, a new collaborative effort was established with the Center for Biofilm Engineering (CBE) at Montana State University, effective June 1, 1996, with the following goal: Return to the originally stated plan of: 1) testing the notion that a particular chemical functionality (or functionalities) could impart special antibacterial properties to certain plasma polymers--as indicated by early, unpublished work at ARC involving a particular fluorine-containing plasma polymer; and 2) of studying the biofilm resistance of plasma polymers derived from a homologous series of fluoroolefins, as well as the biofilm resistance of a series of conventional polymers with varied fluorine content. Although there is a 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. Such work could lead to structure-property relationships in the field of biofilm-polymer interactions.

Four batches of polyethylene (PE) film samples, coated at ARC with plasma-polymerized tetrafluoroethylene (designated PPF4), along with uncoated PE control samples, were supplied to CBE for full biofilm testing against *P. aeruginosa*. Two of the coated batches (BF1 and BF2) had the same thickness of plasma polymer (ca. 115 nm) used in early ARC work and were intended as duplicate sets for developing methodology and checking reproducibility at CBE; the other two coated batches (BF3 and BF4) had different thicknesses (ca. 58 and 230 nm, respectively) for the purpose of verifying prior tentative results at ARC suggesting that coating thickness can have an effect on biofilm resistance—a kind of "substrate effect" similar to one reported in the literature.

To quantify the kinetics and extent of adhesion of *P. aeruginosa* to the coated and uncoated PE films, classical biofilm diagnostic testing at CBE comprised: (1) determination of biofilm net accumulation via destructive sampling and analysis (e.g., total cell numbers, total biofilm protein, and total biofilm polysaccharide per area); and (2) (limited) non-invasive detection of biofilm formation using transmission FT-IR spectroscopy according to the approach developed several years ago at ARC. Because of an FT-IR instrument failure at CBE, with repairs delayed until the '97-'98 academic year, most of the samples obtained from experiments completed at CBE as well as future experiments are being analyzed by transmission FT-IR at ARC.

Following initial calibration work at CBE with the BF1 batch, exposure of samples from the BF2 batch as well as the uncoated PE samples to *P. aeruginosa* over a period of 12 days gave rise to the expected S-shaped kinetic plots for cell counts; however, the PPF4-coated samples reached a plateau with about a 20% higher cell count than did the uncoated samples which conflicted with early ARC results indicating that the coated PE was significantly more biofilm-resistant than the uncoated samples. Paralleling the cell-count plots, plots of biofilm polysaccharide and protein contents for BF2 likewise yielded S-shaped plots, the coated samples reaching somewhat higher levels than the uncoated samples. Limited FT-IR results confirmed the higher biofilm uptake in the coated samples, based on quantitative assessment of the absorbance change at 3300 cm-1 associated with water entrained in the biofilm. A follow-on experiment, involving an accelerated build-up of biofilm over a 60-hour period, yielded nearly identical kinetic plots for the BF2-coated and uncoated PE samples with steady-state plateaus being attained in about 10-20 hours. In replicate experiments involving BF3-coated and uncoated PE samples (the former having the same surface composition as the BF2-coated PE but one-half the thickness of plasma-deposited polymer), no significant difference was seen in the kinetic plots of cell counts for the coated and uncoated samples. Again, this result conflicted with a prior ARC finding that the biofilm resistance of the PPF4-type coating increased (up to a point) with increasing thickness of the plasma-polymer coating. Apart from discarding "preconceived" ideas about the potential biofilm resistance of PPF4 coatings arising from prior ARC work, these new results at CBE lead to the tentative conclusion that, insofar as *P. aeruginosa* culture is concerned, such coatings show no promise in developing the desired antibacterial coatings. Nevertheless, it is planned to carry out some biofilm experiments with at least one particular homologue of PPF4, namely PPF2 representing the plasma polymer of vinylidene fluoride, and possibly also PPF3 representing the plasma polymer of trifluoroethylene. Since the biofilm resistance of plasma polymers in the PPFx series (x = 1-4), when subjected to *P. aeruginosa*, have heretofore not been reported in the literature, it is

desirable to carry out such experiments to the point of preparing a publishable manuscript. Moreover, the transmission FT-IR technique employed in this work is novel and worthy of publication in its own right, and this will be incorporated in a forthcoming manuscript. In particular, the FT-IR spectra of biofilm-covered, coated and uncoated PE films, provide important information regarding the presence of entrained water, proteins and polysaccharides; they also provide direct indications of the heterogeneity or non-uniformity of biofilm layering.

Apart from completing work on PPFx coatings exposed to *P. aeruginosa*, including BF4, there is interest in seeing how such coatings respond to various other common bacteria, e.g., *E. coli*, *S. aureus* and *S. epidermidis*. Additional work in the third and final year of this grant may include examination of biofilm formation on oxygen plasma surface-modified polymers. There are literature indications that some commercial polymers, after being subjected to an rf glow-discharge, air or oxygen plasma, displayed reduced bacterial attachment when exposed to a marine *Pseudomonas* species, apparently as a result of acquiring a hydrophilic character.

In addition to providing various plasma-deposited coatings on PE film for biofilm testing at CBE, basic research at ARC included rf sputtering of polytetrafluoroethylene film using several inert gases to yield polymer-coated substrates. This work, which involved detailed microstructural analyses of the sputter-deposited coatings, provided a background for a convenient alternative to plasma polymerization as a means of obtaining polymeric deposits on different surfaces.

Bacterial cells attach to almost any surface in contact with an aqueous medium. Once attached, the cells grow, reproduce and produce extracellular polymeric substances (predominantly exopolysaccharides) which provide a matrix for a community of trapped, living microorganisms known as a biofilm or microbial film. Biofilms possess either beneficial or undesirable properties depending upon their involvement. Since this research is aimed at biofilm-resistant surfaces, the present discussion of potential Earth benefits is limited to situations involving the undesirable properties of biofilms. An example of such a situation is the costly biofouling of ship exteriors, water pipes, heat exchangers, and various industrial engineering systems promoted by bacterial attachment to all kinds of surfaces-metal, ceramic, plastic, or glass. Another example, in domestic or industrial systems involving humid environments, is undetected biofilm formation in air conditioning units which, under rare and very adverse conditions, could provoke an episode of Legionnaires' disease. Likewise, biofilm formation in the air-circulation ducts of commercial aircraft can expose passengers to potential health problems, while the case of potential biofilm formation in the water reclamation system(s) of long-duration space missions (such as the future International Space Station) has been noted above. In the medical area, nosocomial infections arising from unrecognized biofilm formed on the surfaces of catheters and intravascular devices—infections that often result in fatalities—are quite common. Indeed, biofilm-layered, urinary catheters and attendant urinary tract infections are the major cause of morbidity in hospitalized patients. Other biomedical examples where biofilms can play an unpleasant role are contact lenses and various artificial prosthetic devices. Thus, there are many Earth benefits to be derived from developing biofilm-resistant surfaces or coatings. It is worth stressing that biofilms, once established, resist physical cleaning and penetration by biocides, and their formation must therefore be avoided or suppressed.

FY96 Publications, Presentations, and Other Accomplishments:

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Rapid Bacterial Testing for Spacecraft Water

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Project Identification: 199-04-17-18
Initial Funding Date: 1/96
FY 1996 Funding: $94,923.00

Solicitation: 95-OLMSA-01
Expiration: 12/96
Students Funded Under Research: 3

Task Description:

In water microbiology, there is a need for rapid methods to enumerate specific viable bacteria. This is a particular concern in relation to water which will be reclaimed for potable use on Space Station Alpha. Our principal objective is to develop procedures which will permit the detection of specific marker bacteria which would be used to monitor the performance of the water reclamation and storage systems. In addition, the techniques will be applicable to the detection of particular pathogenic bacteria. Our novel approach (patent pending), which utilizes membrane filtration and combines a fluorochrome for assessment of respiratory activity with specific fluorescent antibody detection of waterborne bacteria, will be evaluated in comparison with molecular methods which will be developed in this project. These include fluorescent *in situ* hybridization following membrane filtration and microcolony formation, to permit rapid quantitation of specific, viable bacteria. Fluorescent *in situ* PCR will also be investigated for sensitive detection of specific bacteria. Results will be applicable not only to spacecraft systems but will also have applications for Earth-based situations. Similar methodologies would be of great value for the examination of clinical and fecal specimens, potable waters, natural waters, foods, and soils, for more timely and reliable detection of specific microbial contamination. Other applications include the examination of purified waters used in the pharmaceutical industry, laboratories, and the electronics industry.

We will develop analytical procedures to identify and quantify bacteria in waste water and product water on spacecraft, permitting more timely measurement and control of bacterial contaminants, and facilitating development of standards and countermeasures to optimize crew health, safety, and productivity.

Efforts have been directed towards the development of a technique that allows the staining of bacteria retained on black polycarbonate filters with two or more fluorochromes to determine, from a single filter: (a) the total number of bacteria present in a water sample; (b) if a specific strain of bacteria is present within the total population; and (c) if these bacteria are viable. The different stains or probes used include: (1) total cell counts using DAPI and acridine orange, (2) identification of specific strains of bacteria using TRITC and FITC labeled secondary antibodies raised against anti-*E. coli* O157:H7 primary antibodies, (3) detection of respiratory activity using CTC, (4) detection of nutrient responsiveness using the direct viable count (DVC) technique, (5) detection of membrane potential using calcofluor white, DiOC6(3), and Rh123, (6) detection of loss of membrane potential using DiBAC4(3), and (7) determination of viability using the commercially available Live/Dead BacLight kit (Molecular Probes, Inc., Eugene, OR). In addition to these assays, agar plate assays for

determination of total viable counts (R2A agar) and lethality and injury within populations of bacteria after exposure to disinfection (TLY and TLYD agar) have been incorporated.

A modified version of the CTC/fluorescent antibody filter assay has been used with *E. coli* O157:H7 cells. Bacteria retained on black polycarbonate filters were incubated with CTC and stained with TRITC labeled fluorescent antibodies, followed by staining with DiBAC4(3). The modified protocol produced promising results. In additon, highly efficient (>90%) bacterial detection by immunomagnetic separation (IMS) and the compatibility of IMS with CTC incubation to determine respiratory activity, using *E. coli* O157:H7 has been achieved. Staining with DAPI or a specific fluorescein-conjugated anti-O157 antibody was used to allow visualization of bacteria by epifluorescence microscopy. TRITC labeled fluorescent antibodies, CTC, DiBAC4(3) and the Live/Dead BacLight kit were tested using *E. coli* O157:H7 cultures after exposure to lethal temperatures and concentrations of formalin, ethanol, and chlorine. All stains and probes evaluated demonstrated consistent and predictable results with disinfected and starved bacteria. Sub-lethal chlorine injury is being evaluated using the same stains and probes listed above, with the addition of Rh123. Injury is also being determined by use of TLY and TLYD plate agars, where TLYD plates contain deoxycholate, which inhibits the growth of injured bacteria.

A ChemScan analysis system (Chemunex, France) has been acquired using Department of Defence (Army) funding. This system includes a dual channel laser that can excite two different fluorochromes or probes simultaneously. It can scan an entire filter (25mm diam.) in less than three minutes, locating and counting cells of specific size and fluorescence (i.e., color). A computerized mechanical microscope stage is used to confirm positive cells.

The techniques we are developing for rapid detection of specific bacteria in conjunction with viability assessment have attracted significant attention among environmental microbiologists. The combination of immunomagnetic separation with the CTC respiration assay and fluorescent antibody staining permits direct detection of bacterial contaminants within six-seven hours. Most other methods employ a 12-24 hour enrichment prior to identification. A patent application on this technology is currently being evaluated. One of the impediments to timely assessment of water and food quality has been the time required to obtain results using traditional or even novel techniques. These procedures will be used for monitoring potable water, foods, and parenteral (injectible) liquids.

Our experiments with disinfection and starvation, using these new methods to detect bacteria, will generate more reliable data on bacterial injury, lethality, and survival.

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Air Quality Monitoring Sensor Using Long Pathlength FTIR

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Project Identification: 199-04-17-23
Initial Funding Date: 5/96
FY 1996 Funding: $115,274

Solicitation: 95-OLMSA-01
Expiration: 4/97
Students Funded Under Research: 0

Task Description:

Monitoring the air in advanced exploration and planetary habitats for chemical contaminants is an analytical challenge. NASA has at its disposal mass spectrometry techniques to identify and quantify some of the contaminants of interest. The problem with MS and IMS is that it is a active point sampling technique. To monitor an enclosed space a sampling system needs to be designed to acquire a representative sample. This project investigates the use of Fourier transform infrared spectroscopy as an ambient air monitoring sensor. The advantage of this technique over MS is that it is a passive area sampling technique.

The program is a laboratory investigation to provide the initial research to implement FTIR as an ambient air sensor. The program is divided into tasks which include developing spectral signatures of the compounds of interest, determining the optimum pathelength for detection, assessing the extent of interferences in mixtures and assessing the best data reduction technique from a set of quantitation methods in current use in spectroscopy. This program is the first step to both a conceptual instrument design and an air monitoring system which is sensitive flexible and can adapt to changing monitoring requirements.

This is a new effort and is based on Arthur D. Little's extensive experience in both monitoring aspects germane to NASA and our extensive experience in vibrational spectroscopy used to solve problems for both commercial and government clients.

The benefits to NASA are a new monitoring technique capable of both quantitative and qualitative analysis. It is able to detect some inorganics in addition to all the organics of interest. In addition, the method is useful for identification of an unknown organic. If an unusual contaminant is present, the FTIR technique can detect and identify this material.

Miniaturized Liquid Chromatography

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Funding:

Project Identification: 199-04-17-17
Initial Funding Date: 12/95
FY 1996 Funding: $73,189

Solicitation: 95-OLMSA-01
Expiration: 11/98
Students Funded Under Research: 2

Task Description:

The effort addressed in this research proposal focuses on the development, testing, and implementation of novel chemical sensor instrumentation for operation in long-term, closed-loop, spacelife support systems. Of particular need is instrumentation for the detection, identification, and quantitation of organic contaminants in water generated by on-board recycling/management systems Such an instrument should also function in an alarm mode in the event of contamination. Thus, this advanced technology development project has strong linkages to both the environmental health and advanced life support needs of the space program.

The proposed research describes the development of a new class of analytical instrumentation. Our strategy couples the well-known capabilities of liquid chromatographic (LC) methods with opportunities for extremes in miniaturization using micromachining techniques to integrate all of the key functional components (i.e., column, detector, pump, and injector) into a "chip-scale" LC instrumental platform. Central to our effort is the creation of a "chip-scale," low voltage pump that can also function as a sample injector for the controlled delivery and metering of fluids. Importantly, the development of a low voltage pumping system addresses a critical need in the eventual realization of "chemical analysis on a chip." As such, the new instrument will represent an extensive size reduction for payload minimization and greatly reduce the consumption of materials as well as the generation of wastes.

The major focus during the first few months of this project has been on the continued development of a "lab-bench" scale fluid delivery system. The basis of this novel delivery system relies on electrochemically-induced changes in the surface tension of mercury in contact with an electrolytic solution. Thus, by harnessing the changes in the heights of mercury in a cell designed for one-way fluid flow, a low power (tens of milliwatts) pumping system can be constructed. Experiments have been conducted that test the effects of the chemical composition and identity of the supporting electrolyte, the magnitude and frequency of both steps in applied voltage and in applied current, and the size and shape of the pump on the rate of fluid flow. Recent results indicate that 1) fluid flow rates as low as a few microliters per minute up to a few thousand microliters per minute can be readily achieved, 2) fluid flow rates can be manipulated by changes in the frequency and the magnitude of the steps in both applied voltage and applied current, and 3) the inertial drag of mercury limits the upper effective value of the frequency change. Experiments to test the consistency of fluid flow and mixing efficiency are underway. An investigation of the types of materials to use as membranes for separation of the pump electrolyte and sample solutions has also been initiated.

Efforts have also been directed toward the design of first generation chromatographic and solution mixing columns in a silicon-glass bimorph structure. This work, which is conducted in collaboration with the microelectromechanical devices group at the Jet Propulsion Laboratory, has focused to date on the design of several possible geometeries for the flow channels, including structures with serpentine and Archimedes' circle-like patterns. Flow channels with a diameter of ~50 micrometers have been targeted for initial performance evaluations. The construction of a prototype electrochemical detector for monitoring fluid flow in the etched silicon channels has commenced.

During the start-up phase of the project, several equipment items have been procured. The most important is a conventional fluid pumping system with a low flow rate. This system facilitates an assessment of the mercury pump by providing a platform for a direct head-to-head comparison of performance. The procurement of a high sensitivity optical microscope for visualizing the movement of mercury within the pump housing and the flow of fluid in the solution channels will soon be completed.

Conventional sampling and analysis techniques often incur substantial labor and equipment costs. Because the number of chemicals falling under drinking water regulations continues to increase, costs will continue to escalate unless new monitoring technologies that operate in a real-time, low-maintenance mode are developed. The proposed chemical analysis instrumentation which can be used in a liquid chromatographic and/or flow injection analysis mode potentially targets this critical need.

To estimate roughly the potential impact of the proposed instrument, it is instructive to assess the costs of conventional sampling and analysis techniques. Although only a beginning, the development of a field-deployable instrument offers a potentially dramatic reduction in monitoring costs. The needs of the water treatment industry for disinfection by chlorine will serve as an example. Currently, the expense of manual testing derives mostly from labor costs. Based on costs incurred by conventional testing laboratories, each manual test conservatively costs ~$3.30. Automatic analyzers, while more expensive than test kits, run ~$2.45 per day. It is projected that the reduction in associated costs using the proposed technology would lead to a 25% reduction in analysis costs when compared to existing automatic analyzers, and a 45% reduction in analysis cost over manual testing.

Even more important to the cost impact of miniaturization is the reduction in volume of spent reagents, samples, and other chemicals for waste handling. Chemical waste disposal costs vary widely depending on the nature of the waste and local disposal regulations. The increasing cost for disposal of chemical waste underscores the need for the proposed analytical systems, which would reduce waste generation. Given the remote location of sampling points, for example, in the above disinfection analyses, the waste generated by an automatic analyzer generally becomes a point-source discharge. A typical batch-flow analyzer, generating 100 milliliters of waste per determination, would produce 20,000 liters of waste per year. This output translates into an estimated disposal cost of ~$10,000 per year, assuming the waste stream is devoid of regulated materials. The cost of disposal could easily exceed $39,000 per year for regulated materials (e.g., heavy metals). Our proposed technology, operating, for example, at fluid flows of five microliters per minute, would generate three liters of waste per year and require 66 years to fill a 200 liter drum. Disposal costs would be between only $62 to $66 per year.

Taken together, the above analysis reveals that the proposed instrumentation has the potential to have a major impact on environmental monitoring, a critical Earth-bound need. This technology also has applications to other important areas, including the on-line monitoring and control of chemical industrial processes. As with environmental monitoring, the majority of process control determinations are conducted in laboratories removed from the sampling points. This situation can incur additional manufacturing costs because of incorrect product formulations as well as from the generation of additional waste.

Pulse Tube Refrigeration New Techniques for Improving Efficiency

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Funding:

Project Identification: 199-80-07-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $141,000
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 FY96 of this contract includes the following areas: (i) Thermal conductance measurements of stacked screens, (ii) theoretical studies of the effect of regenerator conductance on pulse tube performance, (iii)

experimental measurements on the comparison of straight screen regenerators with stepped screen regenerators and etched foil regenerators, and (iv) modeling of inertance tubes to enhance pulse tube refrigerator performance.

Stacked screens are very commonly used as the matrix of regenerators, but the axial thermal conductance of these stacked screens has never been measured. Without such data, the regenerator geometry cannot be fully optimized. We have measured both stainless steel and phosphor bronze screens as a function of porosity and the helium gas fill pressure. For a stainless steel screen, the conductivity degradation factor is found to be about 0.1 compared with 0.3 found from a crude measurement made many years ago. This new value can now be used in more accurate optimization of regenerators.

Our REGEN3.1 model was used to compare the overall pulse tube refrigerator coefficient of performance COP when regenerators were optimized using different conductivity degradation factors. The new degradation factor of 0.1 instead of 0.3 leads to a 10% increase in the COP of the pulse tube refrigerator. This lower factor indicates that the use of Ti6Al4V material for the regenerator tube becomes even more important than original believed.

Several screen regenerators have been fabricated based upon the modeling optimizations discussed above. These regenerators will be used in an experimental pulse tube apparatus to compare the overall performance of the refrigerator with different screen regenerators. These straight regenerators will be compared with the performance of a stepped regenerator. Last fiscal year, we used our REGEN3.1 model to show that stepped regenerators would offer very little improvement over straight regenerators. We also plan to use another model, developed at Los Alamos and based on thermoacoustic theory, to study the stepped regenerator. The experimental measurements on the screen regenerators will also be compared with measurements on an etched foil regenerator.

For pulse tube refrigerators that have more than a few watts of refrigeration at 80 K, the use of a long, narrow tube, known as an inertance tube, between the pulse tube and the reservoir, can bring about desirable phase shifts between the pressure and the mass flow rate. We have modeled this effect and developed a simple analytical procedure to optimize the geometry of the inertance tube. We expect to perform experimental tests with such inertance tubes in the next phase of this program. We also plan to make measurements of tapered pulse tubes to study the possible improvement in pulse tube efficiency caused by the reduced flow streaming in a tapered pulse tube.

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.

Modeling, Monitoring and Fault Diagnosis of Spacecraft Air Contaminants

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Project Identification: 199-04-17-21
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $110,137
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 two-dimensional implementation for monitoring and detection of indoor air contaminants has been completed based upon Implicit Kalman Filtering.

2. A new Source Identification algorithm has been developed and implemented for the convective-dispersion process of contaminant transport.

3. A new coarse sensor algorithm has been developed and applied to the development of an airborne contaminant monitoring system.

4. A three-dimensional flow and contaminant detection algorithm has been developed and implemented. The cabin flow problem is solved using a commercial finite-element package (FIDAP) and the contaminant detection algorithm uses Implicit Kalman Filtering.

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.

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Capillary Electrophoretic Methods for Monitoring Spacecraft Water Quality

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Project Identification: 199-04-11-36
Solicitation: 93-OLMSA-07
Initial Funding Date: 10/94
Expiration: 9/97
FY 1996 Funding: $110,000
Students Funded Under Research: 0

Responsible NASA Center: JSC

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.

What has been accomplished so far? 1) Evaluation of electrolyte chemistries. This task is an ongoing process because new electrolyte chemistries continually appear in the literature or in the market place. To date, over 30 variations of cation and anion electrolytes have been thoroughly tested using a Waters Quanta 4000 laboratory CE. Evaluation of promising electrolytes using a new Hewlett Packard 3D CE system was initiated in FY96. 2) Based on an evaluation of 30+ electrolyte chemistries, three were chosen and optimized toward a list of target anions and cations that appear in the joint U.S./Russian water quality specifications (draft document). For simplicity, direct and indirect UV absorption detection at a single wavelength (214 nm) was used, and separation selectivity and sensitivity were optimized at the expense of analysis time. Results show that 90% of the target ions can be resolved with CE, and 67% of the target ions can be detected at the specified levels by using a 75 cm x 50 μm extended light path (bubble-cell) capillary using the three different separation schemes. 3) Construction of the CE breadboard for KC-135 testing was completed. The breadboard unit, constructed from commercial off-the-shelf components, utilized a laptop computer for data acquisition and 110 VAC power for the UV source and battery power for the capillary and electronics. For simplicity, a fixed 214 nm wavelength from a zinc UV lamp was used, with a shortened bubble-cell capillary. This unit was then subjected to two KC-135 flights, during which tests of both the anion and cation method were conducted. Although problems with baseline noise were encountered, separation of ions was demonstrated. Thus, the objective of demonstrating feasibility was

met. 4) Several bubble exclusion systems were tested during the KC-135 flights, and were found to be ineffective. Work remains to be done in the area of water sample injection with bubble exclusion.

What questions have been answered? 1) We have demonstrated the separation and detection of 90% of the target anions and cations using terrestrial laboratory CE instruments. 2) With a breadboard unit, we have demonstrated the separation of ions in KC-135 microgravity simulations.

What new questions have arrisen? 1) How can we eliminate sources of electronic noise that give rise to baseline noise and drift? 2) How can the sample injection be automated to ensure reproducibility? 3) How can the injection of air bubbles be avoided?
How does this year's progress affect future work on this task? This year's progress represents the completion of a major milestone: The construction and initial KC-135 testing of a breadboard microgravity-compatible CE. Based on the results of KC-135 testing, the breadboard design will be optimized to solve the following problems: eliminate baseline noise in the electropherograms, automate the sample injection process, and eliminate air from the water sample that is injected into the CE. Follow-on ground tests and KC-135 tests of the redesigned breadboard system will be required.

In principle, the separation mechanism in CE is independent of gravity and the methods and procedures developed for flight use can also be adapted for ground use and vice-versa. There are three general sectors that can benefit from the technology being developed by the investigators: the environmental laboratory, the clinical laboratory, and the ultrapure chemical industry. The environmental analytical laboratory can and does already benefit from the products of this research program. CE methods for EPA and NASA-regulated water contaminants have been used to analyze water samples from a variety of ground and space applications. The Water and Food Analytical Laboratory (WAFAL) at NASA/JSC uses these methods routinely for the analysis of drinking water, waste water, and reclaimed water samples. Routinely monitored contaminants fall into three classes: 1) small organic acids and amines, 2) common inorganic anions and cations, and 3) transition metals. CE is both a routine instrument and a niche tool for special or difficult analyses and is capable of low to mid ppb (<μg/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.

Micro-mass Spectrometer for Containment Gas Monitoring

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Co-Investigators:

Funding:

Project Identification: 199-80-04-01
Initial Funding Date: 5/96
FY 1996 Funding: $180,000

Solicitation: 95-OLMSA-01
Expiration: 5/98
Students Funded Under Research: 0

Task Description:

The human missions to the space station and to the planets will be of longer duration than missions undertaken so far. Activities in the habitat and outgassing of materials will release harmful chemicals in the enclosed atmosphere. The small concentrations of toxic chemicals could build up to hazardous levels in long term habitations. It is critical that the habitats of the astronauts be monitored for such chemicals. Development of a sensor to detect small quantities of these toxic/hazardous chemicals is, therefore, needed.

The objective of this proposal is to perform research leading to the development of a small, low power, low maintenance sensor for monitoring the air quality in human exploration and planetary habitats. The sensor will be based on the technologies of a micro-mass spectrometer (m-MS), and an active pixel sensor (APS) array detector. The m-MS uses a miniaturized 180 deflection magnetic sector analyzer designed and developed at JPL. APS technology developed at JPL for photo detection is an ultra low power, low noise imaging sensor technology. The input photodetector of the APS will be replaced with metal strips with high spatial resolution (<25 mm) for direct detection of ions in the mass spectrometer. The combination of the two technologies will produce an instrument with high sensitivity (ppb concentration level) and specificity. The proposed m-MS will be compact, reliable, compatible with microgravity, and have a long operational life. The mass spectrometer will weigh 0.5-0.7 kg, and consume 1-2 W of power.

The development of the proposed instrument will constitute a major technology advancement for an i*n situ* chemical analyzer. It will find other important applications in the measurement of planetary atmosphere and planetary surfaces for future NASA missions (e.g., Discovery Missions, Mars Program). Such a miniaturized mass spectrometer is also much needed for environmental and industrial process measurements.

The task was funded in summer of 1996, and by the time the account was established, it was August 1996. There was not any significant time left in FY96 for the performance of the task.

However, the task is progressing as planned.

The micro-mass spectrometer when combined with a small GC will make a truly portable GC-MS system for on-site, real time measurement of environmental pollutants. At the present time such measurements are generally performed by collecting a sample and sending it to an analytical laboratory. The analysis of contaminated samples away from the site delays analytical results. A large number of such samples are often

found uncontaminated and, hence, the method has proven to be costly. The delay entails inefficient use of manpower and equipment used for characterization and remediation of sites. On-site analysis will overcome this problem. Analysis of indoor atmosphere can be performed by the use of this portable analyzer. Indoor pollution has been recognized to be a major health concern.

The mass spectrometer can be applied to various other measurements which are important for public health (e.g., chemicals at industrial sites, process control, toxic waste sites, workplace, and chemical spills). So far, the unwieldy nature of a mass spectrometer (due to its large size, mass, and power requirements) has limited the use of this powerful analytical instrument to laboratories.

FY96 Publications, Presentations, and Other Accomplishments:

Sinha, M. Micro-mass Spectrometer for Contamination Monitoring. Proceedings of the Advanced Environmental Monitoring Workshop, Glendale, CA, April 23-25, 1996.

Liquid Phase Piezoelectric Immunosensors

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Funding:

Project Identification: 199-04-17-14
Solicitation: 93-OLMSA-07
Initial Funding Date: 3/96
Expiration: 3/97
FY 1996 Funding: $91,801
Students Funded Under Research: 3

Task Description:

Piezoelectric crystal sensors offer excellent sensitivity and design simplicity that make them suited for space technology. The early applications of the respective sensors were limited to measurements in the gas phase. However, subsequent technological advances including immobilization protocols and improvements in oscillator circuit designs that facilitate oscillation in the liquid phase have led to a rapidly rising interest in piezoelectric immunosensors. The overall objective of the project is to develop a piezoelectric immunosensor for *E. coli*, a representative bacteria, using antibodies as coatings. The research plan includes the evaluation of several antibody immobilization techniques, design and evaluation of a suitable oscillator circuit, and the design of a sensor array in conjunction of a flow injection system.

Various oscillator circuits that are capable of allowing crystal oscillations under liquid phase were constructed and tested. After intensive experimentation, one in-house developed circuit was evaluated to be the most reliable circuit. This circuit was then used for all subsequent experiments. Different antibody immobilization methodologies were evaluated including the use of protein A, protein G, and cross-linking via glutaraldehyde. The use of protein A as a precoating of the quartz crystal followed by coating with antibody proved to be suitable for measuring *E. coli*. Current results show that *E. coli* concentrations in the range of 102 cells/ml can be detected. Although precoating with protein G and cross-linking with glutaraldehyde offer high responses, the reproducibility is not yet satisfactory. The effect of temperature and viscosity on the crystal frequency was evaluated and the results showed that crystal frequency decreased with the increase of solution temperature in the range of 30-50°C. A linear decrease in the frequency was noticed as the viscosity increased from 1.002 to 2.25h.

The developed oscillator circuit was extrapolated to result in a multi-oscillator device that has the capability to simultaneously oscillate 4 piezoelectric crystals. This multi-oscillator device is presently being integrated into a Computer Automated Biosensor Array (CABA), which can be used to detect multiple agents or to fingerprint a particular agent in the event of cross-reactivity. This sensor array system (CABA) consists of a) multi-oscillator device, b) a multi-channel frequency counter capable of counting up to 20 Mhz with a 1 Hz resolution, c) computer hardware and software to continuously log the frequency data to a spreadsheet, and d) pattern recognition software to automatically retrieve the frequency data from the spreadsheet and classify or fingerprint the unknown agent based on the previously inputted raw data. Presently a demo version of pattern recognition software (Pirouette Multivariate Data Analysis, Infometrix Inc.) is being evaluated to determine the applicability of the software for the present application. Once the multi-sensor is completed, its performance will be evaluated in conjunction of a flow injection system.

The proposed technology can be adapted to monitor various pathogens that may cause diseases and/or affect the quality of life. Benefits may include possible applications for space, clinical, environmental and food analysis. The successful technology will be useful to several state and federal regulatory agencies. With respect to the CABA, as multiple sensors with different protein and anti-body coatings can be used, the effect of cross reactivity (e.g., attachment of bacteria other than *E. coli*) can be ascertained using the advanced multi-variate data analysis tools. If proven successful, this advancement in fingerprinting technology would not only be an advancement in bacterial detection underwater, but can also be extrapolated to various biosensor techniques that are presently being hampered by cross reactivity.

FY96 Publications, Presentations, and Other Accomplishments:

Attili, B.S. and Suleiman, A.A. Development of piezoelectric biosensors for the determination of cocaine and cortisol. Proc. 19th Annual Meeting of the Adhesion Society, T.C. Ward, ed., Myrtle Beach, SC, P.445 (Feb 18-21, 1996).

Attili, B.S. and Suleimann, A.A. Piezoelectric immunosensor for detection of cocaine. Microchemical J., 54, 174 (1996).

Li, X., Fortuney, A., Guilbault, G.G., and Suleiman, A.A. Determination of bilirubin by fiber optic biosensor. Anal. Letters, 29(2), 181 (1996).

Suleiman, A.A. Liquid phase piezoelectric immunosensors. Proc. NASA Advanced Environmental Monitoring and Control Program Workshop, Glendale, CA (April 23-25, 1996).

Suleiman, A.A. and Guilbault, G.G. "Piezoelectric and surface acoustic wave sensors" in "The Handbook of Chemical and Biological Sensors." Edited by: Taylor, R.F. and Schultz, J.S. IOP Publishing, pp 483-494, (1996).

Suleiman, A.A. and Kornhauser, S.H. Piezoelectric immunosensors. Amer. J. of Electromedicine, 100, (1996).

Multigas Sensor for Advanced Life Support

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Funding:

Project Identification: 199-04-17-20
Initial Funding Date: 11/95
FY 1996 Funding: $104,001

Solicitation: 93-OLMSA-07
Expiration: 11/97
Students Funded Under Research: 1

Task Description:

During the period covering FY 1996, substantial progress has been achieved in the project "Multigas Sensor for Advanced Life Support" using novel nonaqueous electrolyte electrochemical sensor. Experimental setup and techniques have been developed. Suitable electrode materials and electrolyte solutions have been prepared and experiments conducted with a three electrode system in a three compartment cell using electrochemical instrumentation interfaced with a computer to run experiments, data collection and data reduction.

Nonaqueous electrolytes based on aprotic solvent mixtures and tetraalkyl ammonium salt and gold sensing electrode with platinum counter and silver quasi reference and/or platinum pseudo reference electrode have been used in the cell in a dry box and/or in a dry bag with a positive pressure of argon. Using the laboratory cell and laboratory electrochemical instrumentation, cathodic linear scanning experiments have been conducted with known concentrations of oxygen, carbon dioxide and moisture. Well defined current peaks at characteristic potentials have been obtained and for oxygen at -85 V, for carbon dioxide at -2.3 V and for water vapor at about -1.8 V vs Ag reference. Experiments have also been conducted with oxygen and water vapor combinations with good detection capability for both species to prove Multigas detection capability of this sensor.

For the detection of volatile organic compounds(VOCs), it is found that most of these compounds undergo electrochemical oxidation much more easily than the reduction on platinum sensing electrode surface. Using platinum sensing electrode and platinum counter electrode with anodic scanning voltage technique from 0 to +2.5 V vs Ag or platinum reference electrode, successful experiments have been carried out. Concentrations of the organic compounds detected are in the range of 200 PPM unless mentioned otherwise. Well defined current peaks are obtained for n-Butanol at 1.6 V vs pt, chloroform at 1.85 V vs pt, dichloromethane at 1.6 V vs pt, carbon tetrachloride at 1.7 V vs pt, i-propanol 1.7 V vs pt and 1.8 V vs Ag. Experiments on organics with both pt and Ag reference showed that these species are easily distinguished from their voltages with proper reference electrode. With tetrachloroethane well defined current peak is obtained at 1.5 V vs pt and 1.7 V vs Ag, trichloroethane gave current peak at 1.8 V vs pt and 1.65 V vs Ag. Ethyl acetate gives current peak at 1.6 V vs pt and 1.8 V vs Ag. For methanol a large peak at 1.9 V vs pt and at 2.15 V vs Ag, for ethanol a peak at 1.8 V vs pt, and for methylethyl ketone a current peak at 1.65 V vs pt and a small peak at 2 V vs Ag are seen.

Some of the results of this project have been presented at the NASA Advanced Environmental Monitoring Workshop held at Glendale, CA, April 23-25, 1996 and published in the Workshop Proceedings.

This unique electrochemical sensor technology based on nonaqueous electrolytes has the potential for long life, low cost and sensitive and reliable performance with multigas/vapor detection and monitoring.

This sensor has applications for monitoring toxic gases and vapors particularly volatile organics (VOCs) at homes and at work place. The sensor has applications in environmental monitoring, in industrial process monitoring and control and in medical diagnostics for blood gas monitoring.

FY96 Publications, Presentations, and Other Accomplishments:

NASA Advanced Environmental Monitoring Workshop, Glendale, CA (April 23-25, 1996).

Water Purification in Microgravity by Freeze Separation

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 199-61-17-02
Initial Funding Date: 2/95
FY 1996 Funding: $137,817

Solicitation: 93-OLMSA-07
Expiration: 2/97
Students Funded Under Research: 0

Task Description:

Water purification by freeze separation 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 objectives of the first phase of Water purification in Microgravity by Freeze Separation were: 1) to determine energy and fluid transport parameters for simulation and prototype development; 2) to demonstrate the ability to create ice crystals with minimum occlusions; and 3) to quantify the thermodynamic separation efficiency of a gravity independent crystallizer.

Three binary aqueous solutions were used to simulate waste streams: sodium chloride, ethanol, and ammonia. The ability to operate and model this process was demonstrated. Single pass separation efficiencies of greater than 99% were realized for ammonia, between 55% and 76% for sodium chloride and up to 68% for ethanol. The sodium chloride test showed that there was no performance degradation as the solute concentration increased from 5 to 20 weight percent.

The next phase of the project focuses on the development of a continuos flow crystallizer. Thermodynamic separation efficiency will again be addressed with a greater emphasis on crystal formation rates. The initial experiment design is complete with acquisition and build phase to commence shortly.

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 1996 Funding: $
Students Funded Under Research: 0

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

The overall objective of this project is the parametric evaluation of a Steam Gasification and Reforming process as a candidate process for detoxifying and recycling biologic and other organic wastes. The intent of the Steam Gasification and Reforming process is to oxidize organic wastes to the inorganic carbon oxides, water 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.

Steam gasification at 1200 - 1400°F with as little as 2% - 5% added oxygen greatly facilitated conversion of the model solid waste compounds into gaseous effluents amenable to catalytic steam reformation. Model waste compounds were tested individually and in combination to simulate the wastes anticipated within enclosed habitat environments. Results obtained indicated that even very low oxygen concentrations (< 3%) were sufficient to suppress the formation of a carbonaceous residue in the gasification reactor.

In the reforming reactor, the use of a nickel/cobalt catalyst at reaction temperatures of 1600 - 1800°F effected efficient destruction of the organic species evolved from the gasification process. These effluents included methane and ammonia. Steam reformation with the nickel/cobalt catalyst also eliminated the noxious odors normally associated with the gasification of butyric acid methionine, plastics, or other organic reagents representative of space waste streams.

In summary, the feasibility has been established for the process of reacting a variety of model waste material with as little as 2% - 5% oxygen in steam to facilitate char reduction and convert model wastes to primarily ash, carbon dioxide, and water.

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 process 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).

The subject Steam Gasification and Reforming process, probably with 5 to 10% added oxygen to reach a point of thermal neutrality, also has the potential of processing biological and/or chemical wastes back into innocuous inorganic compounds on Earth or in remote stations (e.g., 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 incineration process) since it can participate in the oxidation reactions, generating hydrogen, and cannot participate in the production of toxic nitric oxides.

FY96 Publications, Presentations, and Other Accomplishments:

Birbara, Philip J. Advanced waste management technology evaluation. NASA sponsored ALS meeting on "Requirements Definition and Technology Development Needs" October 28, 1996, Houston TX.

Crop Production Optimization Using CO_2 Gas-exchange

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 199-61-17-08
Initial Funding Date: 4/95
FY 1996 Funding: $150,962

Solicitation: 93-OLMSA-07
Expiration: 3/98
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.

Accomplishments.

Based on number of publications, 1996 was the most productive year in the history of this project. We published six papers and nine additional papers are in press. Publications are not the only measure of productivity. The PI visited all three of the NASA field centers involved with crop production studies for advanced life support during 1996 to coordinate research efforts. In March 1997, the PI presented an invited seminar to the biomass production group at Kennedy Space Center on our studies with CO^2 gas exchange. Specific accomplishments are as follows:

1) We conducted three studies in our 10-chamber gas exchange system. These studies characterize soybean productivity over a wide range of temperatures and quantify the decreased productivity that can occur as temperatures deviate from the optimum (Dougher and Bugbee, 1997).

2) We published a major paper on the effects of super-optimal CO_2 on wheat. These studies show that super-optimal reduces yield, but the decrease is only 15 to 20%. We examined the mechanism responsible for the CO_2 effect and found that, contrary to our hypothesis, ethylene does not appear to be involved. The detrimental effect appears to be mediated by reduced rate of respiration (Grotenhuis and Bugbee, 1997).

3) Based on our data, we published a first generation transpiration model for crops in Advanced Life Support (Monje and Bugbee, 1997).

4) In April 1997, we released a new wheat cultivar developed specifically for controlled environments (Bugbee et al. 1997). The cultivar, named "USU-Apogee" outyields the previous best cultivars by 15 to 30%. Both KSC and JSC have reported significantly increased yields through the use of this new cultivar.

Proposed Work.

1) We propose to examine the effects of blue light fractions on cell enlargement, leaf size, radiation capture, and yield in soybeans. We will also use CO_2 gas exchange to quantify soybean yield determinants over the life cycle.

2) We propose to examine the hypothesis that the detrimental effects of CO_2 occur only during a 10-day period of anthesis in wheat. This will be done in a recently built 12 chamber gas exchange system that allows multiple small plots for replication and randomization of treatments.

3) We propose to refine and validate our transpiration model. Specifically, we propose to extend the measurements from wheat to soybeans. Soybeans are representative of many dicotyledenous crop plants that are now used in advanced life support.

4) In the process of developing the cultivar "USU-Apogee," we discarded shorter cultivars because they had slightly reduced yields. However, there is a tremendous demand for an improved super-dwarf cultivar for use in space biology. We are now reselecting among our advanced breeding lines for high yield and extremely short height (20 to 35 cm tall).

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.

FY96 Publications, Presentations, and Other Accomplishments:

Bugbee, B. "Growth analysis and yield components" in "Units, Symbols, and Terminology for Plant Physiology." Edited by: Salisbury, F. Oxford University Press, 1996.

Bugbee, B. and Doucette, W. (abstract) Design of closed chambers for measuring phytoremediation: Optimizing plant growth and chemical mass balance. Hazardous Substance Research Center Annual Meeting Abstracts Book. 1996.

Bugbee, B., Droter, M., and Monje, O. (abstract) Infra-red thermometry: Accurate measurement of leaf to air temperature gradients in CELSS. 1996 COSPAR meeting.

Bugbee, B., Droter, M., Monje, O., and Tanner, B. (abstract) Accuracy and calibration of two commercial infra-red temperature transducers. Agronomy Abstracts, (1996).

Bugbee, B., Koerner, G., Albrechtsen, R., Dewey, W., and Clawson, S. (abstract) USU-Apogee: A new high-yielding dwarf wheat cultivar for life support systems. 1996 COSPAR meeting.

Bugbee, B., Monje, O., and Tanner, B. Quantifiying energy and mass transfer in crop canopies. Advances in Space Res., 18, 149-156 (1996).

Doucette, W. and Bugbee, B. (abstract) Relationship between aerobic biodegradation rates, chemical structure, and soil type for selected petroleum hydrocarbons. Hazardous Substance Research Center Annual Meeting Abstracts. 1996.

Dougher, T. and Bugbee, B. (abstract) Blue light inhibits internode elongation, growth, and yield in soybeans. 1996 COSPAR annual meeting.

Grotenhuis, T., Reuveni, Y., and Bugbee, B. (abstract) Super-optimal CO_2 reduces wheat yield. 1996 COSPAR annual meeting.

McKeehen, J.D., Mitchell, C.A., Wheeler, R.M., Bugbee, B., and Nielsen, S.S. Excess nutrients in hydroponic solutions alter nutrient content of rice, wheat, and potato. Advances in Space Res., 18, 73-83 (1996).

Monje, O. and Bugbee, B. Characterizing photosynthesis and transpiration of plant communities in controlled environments. Acta Hort. ISHS, 123-128 (1996).

Smart, D., Ritchie, K., and Bugbee, B. Mass transfer in the biological fast lane: High CO_2 and a shallow root-zone. Life Support & Biosphere Sci.., 3, 43-46 (1996).

Stahl, R., Greenhalgh, M., Grossl, P., and Bugbee, B. (abstract) Is the pH buffer MES phytotoxic? Agron. Abstr., (1996).

van Iersel, M. and Bugbee, B. Phytotoxic effects of benzimidazole fungicides on bedding plants. J. Am. Soc. Hort. Sci., 121, 1095-1102 (1996).

Volk, T., Tubiello, F., and Bugbee, B. (abstract) Testing the CERES-wheat model with growth chamber data under elevated CO_2. Agron. Abstr., (1996).

Power Assisted Space Suit Joint

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Funding:

Project Identification: 199-06-17-04
Initial Funding Date: 7/96
FY 1996 Funding: $187,112

Solicitation: 95-OLMSA-01
Expiration: 12/96
Students Funded Under Research: 1

Task Description:

The continuous development of Extravehicular Activity (EVA) spacesuit gloves has led to an effective solution for performing extravehicular activity to date. Some limitations of the EVA gloves have been noted that potentially affect productivity in the form of limited dexterity and high torque joints that accelerate the onset of fatigue (Lapham, 1994). The limitations of the current gloves in conjunction with the fact that the mission envelope is currently expanding via task complexity, frequency, and duration, indicate the need for further development of spacesuit gloves.

The spacesuit gloves have evolved over years of development. The emphasis of this development has been on the advancement of a passive restraint and mobility system. The development of advanced materials and processes has led to steps forward in this technology but no leap has been realized. Technology is now available to make a leap in performance capability which will improve astronaut effectiveness during EVA. This increased performance will also reduce mission costs by reducing crew fatigue and increasing mobility, thus increasing task speed and mission duration.

By utilizing technology from the current spacesuit glove in conjunction with state-of-the-art robotics technology, a power-assisted spacesuit glove joint can be created to greatly improve glove performance. The power-assisted joint will only provide enough force to offset the resistance of the pressurized glove joint itself and will not provide strength augmentation to the hand. In this approach, nude body performance will be approached via tuning of the power assist system. Of principal interest will be the improvements in the areas of dexterity and fatigue, but the technology will also address factors involving tactility, range of motion, and comfort. By effectively applying these technologies a synergism will be created that will allow the EVA crewmember to maintain a "hands on" approach that will enhance mission effectiveness by keeping the most important element of the EVA, the crew member, at the worksite.

In fiscal year 1996, ILC Dover and the University of Maryland completed Phase I of the development of the Power Assisted Space Suit Glove. The power assist concept shows merit based on previous studies which have

shown that space suit gloves are the limiting factor during EVA. The approach is to counteract the joint torque in the glove's metacarpalphalangeal (MCP) joint by applying a force exactly equal to that created in the joint due to pressure and velocity induced loads. This approach theoretically will cause the MCP joint performance to mimic nude hand performance. The concept is divided into two technology development areas. The first involves system trade studies and glove softgoods design; ILC Dover is concentrating its efforts on this portion of the project. The second research area is the actuation system design and software development, which is the responsibility of the University of Maryland.

ILC Dover traded off several MCP joint designs and selected a "half" rolling convolute joint design, a modified form of a full rolling convolute design. Load vs. deflection testing showed that the half rolling convolute design had the potential to have the lowest torque of the MCP designs considered. Additionally, this joint design proved to be favorable due to the minimum amount of hardware, and subsequent stand-off required along the thumb/index finger grasping area. After the design trade-off efforts, Phase I activities focused on reducing the torques (and thus loads on the actuation system) and hysteresis seen in the selected half rolling convolute design.

The University of Maryland performed trade studies to select the range of motion sensors, system model, actuation system, and control software algorithms. System modeling was performed based on a one degree of freedom joint which takes into account angular position, inflated restraint stiffness, and joint actuation velocity to model the stiffness and predict the behavior of the MCP joint. An optical encoder, which provides feedback on the position of the actuation cable running to the MCP joint, was selected to provide feedback of the MCP joint's position. A brush-commutated, DC servo motor was selected to provide the counteractive forces needed to negate the torques seen in the MCP joint. A software algorithm was developed to mimic the load vs. deflection data generated during test of the MCP joint. The algorithm takes into account direction of joint movement and velocity of joint movement, both of which affect the load required to counteract the torque seen in the MCP. The glove MCP joint was linked to the actuation system via a low elongation, Spectra cord.

Future activities will include refinement of the MCP joint design to reduce torque and hysteresis, reduction of profile in the actuation system, development of improved software algorithms to better mimic nude hand performance, and ultimately, a manned evaluation shall be performed to quantify the benefits of the Power Assisted Glove system. Performance of the Power Assisted system shall be compared to the performance of the Space Shuttle Space Suit Gloves currently being used.

The current research into power assisted space suit gloves, specifically the MCP joint, is meant to first demonstrate this technology in one area of immediate need. In the future, this technology could be applied to other space suit joints such as the glove's carpometacarpal joint, wrist joint, shoulder joint, and hip joint. Application of the technology in these areas would reduce user fatigue, increase mobility, and even augment strength. These aspects of space suit design will be imperative for future Lunar and Martian exploration missions.

In addition to the above mentioned space applications, the power assisted glove technology could be beneficial in several other industries, including the medical and commercial entertainment industries. The power assist system, coupled with the appropriate sensors, could be used to facilitate movement of prosthetic devices or appendages of persons with loss of mobility. Virtual reality technology could benefit by using force feedback suits to enhance sensory feedback in the virtual reality environment. In the ergonomics and rehabilitation fields, the power assist glove could be used to perform ergonomic evaluations of persons suffering from compromised mobility and reduced strength in their hands or limbs. In addition, the power assisted glove could be modified to provide force feedback, thereby acting as a rehabilitation device.

AI Software Development for Advanced Life Support

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Funding:

Project Identification: 199-61-23-17
Initial Funding Date: 2/95
FY 1996 Funding: $96,000

Solicitation: 93-OLMSA-07
Expiration: 2/98
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.

Work continued on the project though the progress was slower than planned due to reduced funding. Data validation software was completed, support was provided to KSC's selection of Control and Monitor Unit (CMU) software for ALS Breadboard Facility (ABF) monitor and control, and work was begun on modeling ABF data as another step towards building model-based control as discussed in earlier work. (Drysdale, A.E., McRoberts, M.D., Sager, J.C., and Wheeler, R.M. (1992b). Object-oriented, Model-Driven Control. COSPAR, Washington DC. Printed in *Adv. Space. Res.* (1994), 14, (11) 313-322.) The components for the comprehensive data system identified in this paper are either developed or under development.

This year, the data validation package was completed and has been in use by the ABF personnel to validate 19 data streams of computer data collected in ongoing growouts. The data validation module takes the raw ABF data stream and flags anomalies to the operator for disposition. As experience is developed in classifying anomalies automatically, we anticipate that an increasing number of anomalies will be dispositioned without operator intervention. The module takes data from the ABF Oracle database as directed by the operator, flags anomalies, classifies them by type, and the operator dispositions them.

Software to replace the existing monitor and control software, UNDACE (Universal Networked Data Acquisition and Control Engine), has been investigated in collaboration with NASA KSC and Dynamac. UNDACE is a custom software package that provides the necessary functionality for current control and monitor functions, but is a purpose-built system that is no longer supportable (Little and Drysdale, 1996). It needs to be replaced to enhance supportability and provide expanded functionality. The Control and Monitor Unit (CMU) is being

developed for use as the International Space Station (ISS) Test Checkout and Management System (TCMS). Thus it will be supportable for many years and synergistic development is ongoing, making CMU a highly credible system for replacing UNDACE.

An existing model, OCAM-2, was used to evaluate ABF system status. OCAM-2 is a system-level model that currently uses experimental crop data to predict day-to-day performance of a bioregenerative life support system. It runs on G2, a commercially available modeling package, on a variety of workstations. OCAM incorporates all components of an advanced life support system, including functions that are not currently implemented in the ABF, such as the crew component. The general ALS model was modified to make it represent the ABF more closely, and ABF data was imported to drive the model. A data bridge was needed to allow it to work with on-line ABF data. A suitable bridge was developed as part of another project (SuperMOCA), and modified to handle ABF data. The ABF monitor and control system collects data every second. This data is sampled and archived as five-minute data. Five-minute data for the data streams is polled at intervals by a C program and imported into G2, where this data is smoothed and summarized to generate daily data. The daily data is input to the model. Thus, for example, plant growth is driven by CO_2 uptake. Water uptake and plant transpiration in the model is driven by the measured condensate collection. The model calculates system state data from data which is accessible to measurement. Thus, for example, carbon uptake is derived from pCO_2 and CO_2 mass flow data. Plant growth is derived from carbon uptake. Not all data is continuously available. Thus, plant growth is predicted based on the carbon uptake, but is measured at harvest. Harvest data is used to update predicted plant growth data for a run that exceeds one harvest.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Drysdale, A.E. (presentation) Performance measurements and bioregenerative life support system performance: How close are we to achieving cost effectiveness? 31st COSPAR, session F4.5 (1996).

Drysdale, A.E. and Sager, J.C. A re-evaluation of plant lighting for a bioregenerative life support system on the moon. 26th ICES, SAE paper 961557 (1996).

Drysdale, A.E., Fortson, R.E., Sager, J.C., Wheeler, R.M., Stutte, G.W., and Mackowiac, C.L. Reliability of biological systems based on CBF data. 26th ICES, SAE paper 961489 (1996).

Hunter, J. and Drysdale, A.E. Concepts for food processing for lunar and planetary stations. 26th ICES, SAE paper 961415 (1996).

Hunter, J. and Drysdale, A.E. Optimization of food processing for a lunar base. 26th ICES, SAE paper 961413 (1996).

Hunter, J., Steinkraus, K., and Drysdale, A.E. Value of fermented foods for lunar and planetary stations. 26th ICES, SAE paper 961416 (1996).

Little, W. and Drysdale, A.E. The automated control and monitoring of advanced life support systems. 26th ICES, SAE paper 961557 (1996).

Sager, J.C. and Drysdale, A.E. (presentation) Concepts, components, and controls for a CELSS. International Society of Agricultural Engineers, Japan (1996).

Stutte, G.W. and Drysdale, A.E. To grow or not to grow: Decision points in developing life support systems for long duration space missions. Publications of Society of Logistics Engineers. Florida Log '96. Proceedings of the 3rd Annual Technical Conference and Workshop (1996).

Adsorbed Carbon Dioxide and Water Interactions and Maintenance of Low CO_2 Levels in Closed Environments

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Funding:

Project Identification: 199-61-12-12
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $ 125,000
Students Funded Under Research: 0

Responsible NASA Center: ARC

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 second year of the grant, work proceeded along several lines as planned. These include adsorption experiments, process design, and process simulation. The following are the principal accomplishments in each of these areas:

Adsorption Experiments

• Single component isotherms for carbon dioxide were obtained on various organic and inorganic sorbent materials. This data will be used in conjunction with the multi-component experiments presently in progress to analyze the chemistry of co-adsorbed CO_2 and H_2O.

• An apparatus for obtaining multi-component adsorption equilibria was designed, constructed, and tested; the apparatus is presently generating data for the study.

• Column dynamics experiments for the separation of CO_2 from humid air were performed on a variety of materials; one material in particular stands out and we are investigating a new process design based on this material (see below).

Keeping in mind that the main problem associated with closed-loop CO_2 removal from spacecraft air (i.e., no CO_2 or water dump) is one of high power consumption, and that most of the power is used for regenerating spent desiccant beds, we developed a process that eliminates or dramatically reduces the size of the desiccant beds while apparently maintaining CO_2 removal capacity. The process is based on the utilization of a membrane desiccator that removes 90% of the water from the incoming process air stream, and the use of a sorbent that will tolerate water (i.e., CO_2 sorption capacity is not lost) at a water dewpoint of –20°C. The process produces a dry, CO_2 free air stream that passes back on the opposite side of the desiccating membrane, providing the driving force for the desiccation. We anticipate that the process will use approximately 20% of the power required by current technology for removing the same amount of CO_2.

We have discussed the concept with life support engineers at NASA Johnson Space Center and are testing the concept in the laboratory.

Work continues on the development of a new, high efficiency/high accuracy computer algorithm for simulation of multi-component adsorption processors; an article has been written and accepted for publication.

Carbon dioxide buildup is a potentially critical problem for maintaining breathable air in any closed environment. These environments include not only spacecraft and future planetary habitats, but modern buildings that draw in minimal fresh air for reasons of energy savings, passenger aircraft, vehicles on battlefields (such as tanks, helicopters, and personnel carriers), and underwater and high-altitude vehicles. If 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.

FY96 Publications, Presentations, and Other Accomplishments:

DeVantier, B.A. and Finn, J. E. Discrete dispersion relations and Taylor-Galerkin FEM for transport of dispersed fronts. Int. J. Fluid Mech., (1996).

Mohamadinejad, H., Knox, J.C., Finn, J.E., and Smith, J.E. Hardware-independent mathematical and numerical modeling of a four-bed molecular sieve - part 1, Paper No. 961405. 26th International Conference on Environmental Systems, Monterey, CA. July 1996.

Enhanced Molecular Sieve CO_2 Removal Evaluation

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-61-17-11
Initial Funding Date: 1/96
FY 1996 Funding: $165,287

Solicitation: 93-OLMSA-07
Expiration: 1/98
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 of the molecular sieves offer the potential of high payoff for future manned missions by reducing system complexity, weight (including consumables), and power consumption in comparison with competing concepts. The 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 FY96, the first phase of the Enhanced Molecular Sieve CO_2 Removal NRA (Sept. 95 - Sept. 96) was completed, and the second phase (Sept. 96 - Sept. 97) was initiated.

The tasks performed include the following:

Phase 1

- Pump-down curves
- Packing density
- Characterization of sorbent materials — CO_2 capacity in dry and humid air stream
- Full-scale testing — saturation and rapid cycling at different conditions

Phase 2

- Initiated test rig upgrades (sensors, line size, heater tape and insulation, new sorbent bed, replumbing for isothermal and thermally coupled testing)
- Initiated review of analytical performance model and initiation of effort to upgrade and adapt to CO_2 removal with chosen sorbent materials

- Saturation curves for CMS materials
- Packing density and pressure drop data for new sorbent materials

The primary goal of this research is to investigate a new technology for the selective removal of CO_2 from air (or oxygen) for space applications.

Selective CO_2 removal also has terrestrial applications, notably for food storage, production plant cleanup, and submarine air revitalization. Additionally, CO_2 removal 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.

The most important gains of this study may be to facilitate man living in a closed environment by characterizing new CO_2 selective sorbents under different conditions with different bed designs and operations.

FY96 Publications, Presentations, and Other Accomplishments:

MacKnight, A.K. Enhanced molecular sieves for CO_2 removal - Phase I. NASA Advanced Life Support Workshop held at Johnson Space Center (October 28-30, 1996).

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 1996 Funding: $73,338
Students Funded Under Research: 5

Task Description:

This research involves moving 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.

Three major accomplishments were made: a) We have discovered that geometry has a substantial impact on the total mass transport as well as the separation. For example, a multiple annular circular geometry with concentric compartments was compared with an annular geometry with compartments and consequently less boundaries. Both had the same cross sectional flow area and the same outer dimension, and we concluded that more boundaries gave better separation at the expense of mass transport and so a trade off occurs between degree of separation and total amount that can separated in a given time; b) We have discovered, contrary to initial expectation, that species with smaller diffusion coefficients actually transport much faster than those with larger diffusion coefficients. This startling result begins to make sense when we observe that faster species diffuse quickly to the boundary region where the flows are slowest thereby limiting the transport; and c) The equipment that we have built has been used to verify the theories of enhanced mass transport by diffusion and dispersion by oscillatory flows.

Our future work will require a continued evaluation of geometry based in accomplishment (a) above and the evaluation of optimum feed concentrations based on accomplishment (b).

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.

FY96 Publications, Presentations, and Other Accomplishments:

Narayanan, R. The effect of boundaries on enhanced mass transfer in oscillatory flow. Amer. Phys. Soc., Div. Fluid Dynamics, Syracuse (1996).

Narayanan, R. The effects of frequency and geometry on convective mass transfer via oscillatory flow in cylindrical tubes. Amer. Inst. Chem. Eng. Annual Meeting, Chicago (1996).

Poplasky, M.S. Enhanced diffusion in the annular space between oscillating concentric cylinders. M.S. Thesis (under the direction of R. Narayanan), Univ. of Florida (1996).

Testing an Algae-Based Air-Regeneration System Designed For Use in a Weightless Environment

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-61-17-10

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/97

FY 1996 Funding: $0

Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

The major objectives of this project are the determination the basic parameters of the growth of selected subaerial algae on ceramic tubes and the evaluation of their ability to remove CO_2 from the air. At this point we have standardized the culture conditions and completed the initial testing of three strains of algae: the green alga *Chlorella vulgaris* (UTEX 259); *Stichococcus sp.*, a subaerial green algae isolated from southeastern Georgia; and *Gloeocapsa sp.*, a subaerial cyanobacterium also isolated from southeastern Georgia. The algae are initially grown on agar and then suspended in liquid medium and painted onto ceramic tubes. The tubes are incubated in groups of four in polypropylene boxes under a 16:8 light:dark cycle; the photon flux in the box is about 35 $\mu mol\ m^{-2}\ s^{-1}$ (PPF); the total volume of a box containing four tubes is under 600 ml. A minimal salts medium (BBM) is circulated through the tubes for about 30 minutes a day. Under these conditions, visible growth appears on the surface of the tube about one week after inoculation, and heavy growth about two to three weeks later. With periodic changes of the medium in the reservoir, heavy growth can be maintained on a tube for more than 150 days. Under standard conditions (photosynthetic photon flux 35 $\mu mol\ m^{-2}\ s^{-1}$, initial CO_2 concentration about 450 ppm), 80-day-old tubes of *Chlorella vulgaris* remove about 4.8 mg of CO_2 per tube per minute (54 $mg\ m^{-2}\ h^{-1}$). The rate of CO_2 removal increases with the age of the tube, up to at least 100 days. The rate of removal increases linearly with respect to light up to a photosynthetic photon flux of at least 70 $\mu mol\ m^{-2}\ s^{-1}$; it appears that light saturation may begin at photon fluxes as low as 120 $\mu mol\ m^{-2}\ s^{-1}$. The results obtained using *Gloeocapsa* and *Stichococcus* were comparable.

During the second year, we will focus on two main areas. First, we plan to increase the sensitivity of our CO_2 monitoring system by improving our air handling system. This will allow us to use fewer tubes for each set of measurements, which will, in turn, enable us to test a greater variety of algae at the same time. As part of these

improvements we plan to add the capability of testing the rate of CO_2 uptake at higher concentrations of CO_2, more like those encountered in spacecraft. Second, we plan to investigate the effects of increasing the light level. The measurements obtained using *Chlorella* and *Gloeocapsa* indicate that our standard conditions are far below saturating levels of light and that we can easily double the rate of CO_2 uptake by increasing the photosynthetic photon flux.

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.

Biophysical, Mathematical Models of Gas Phase Formation

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Funding:

Project Identification: 199-70-31-20
Solicitation: 93-OLMSA-07
Initial Funding Date: 1/95
Expiration: 1/98
FY 1996 Funding: $73,798
Students Funded Under Research: 2

Responsible NASA Center: JSC

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. 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 is an interesting addition which is being pursued and is being considered for combination with the variable diffusion barrier. Relationships between this model and that of Van Liew and Burkard are currently being explored. Modifications include the stochastic addition of nucleation/growth sites both prior to and during the depressurization. Analysis of gas washout with exercise has indicated that blood perfusion increases several fold, transforming a 360-minute compartment into a 100-minute one ("effective" tissue half-time). We have derived a simpler, but complete, equation starting from the Fick equation and the equation developed by Hlastala, Van Liew, and Burkard. Ours was derived by Srini Srinivasan, Ph.D. To this calculation method, we have added two principles to form what is a new concept in barophysiology, viz, the "Limited-Lifetime, Distributed Radii Density" (LL/DRD model). It states that: a) micronuclei are limited in their lifetime; they do not posses the lifetimes of weeks to months envisioned by E.N. Harvey and described by him in the 1940s. Nuclei lifetimes have been measured by us and are on the order of hours; and b) micronuclei are distributed in their radii and relative densities; modifications of this can cause effects in response to surface tension and relaxation.

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 30,00 feet.

This work describes a process that affects individuals on Earth as well as astronauts. It will lead to the development of new types of decompression tables that will mitigate decompression sickness and can be applied to earth-based decompressions as well. The work has described a new and rational basic understanding of the cellular mechanisms behind decompression sickness.
This information would also be of value for both SCUBA and commercial divers, especially those involved in the recovery of deep-sea oil. The benefits to commercial diving should be substantial by the implementation of certain aspects of this work.

FY96 Publications, Presentations, and Other Accomplishments:

Conkin, J., Foster, P.P., Kumar, K.V., Powell, M.R., and Waligora, J.M. Characterization of venous gas emboli in the pulmonary artery of humans after hypobaric decompressions. Undersea Hyperbaric Med., 23 (Suppl.), 28 (1996).

Conkin, J., Foster, P.P., Powell, M.R., and Waligora, J.M. Relationship of the time course of venous gas bubbles to altitude decompression illness. Undersea Hyperbaric Med., 23, 141-149 (1996).

Conkin, J., Jumar, K.V., Powell, M.R., Foster, P.P., and Waligora, J.M. A probability model of hypobaric decompression sickness based on 66 chamber tests. Aviat. Space Environ. Med., 67, 176-183 (1996).

Foster, P.P., Shiau, L.J., Conkin, J., Powell, M.R., Chhikara, R.S., and Waligora, J.M. (abstract) Influence of decompression procedure on the metabolic gas participation to bubble formation in hypobaric exposures. Aerospace Med. Ass. annual meeting, Abstract No. 241, pp A39 (1996).

Gerth, W.A. and Vann, R.D. Importance of tissue elasticity in statistical bubble dynamics models of DCS risk. Undersea and Hyperbaric Med., 22 (Supplement), 69 (1995).

Gerth, W.A. and Vann, R.D. Probabilistic gas and bubble dynamics models of decompression sickness occurrence in air and N_2-O_2 diving. Undersea and Hyperbaric Med., (in press).

Kumar, K.V. and Powell, M.P. Survivorship models for estimating the risk of decompression sickness. Aviat. Space Environ. Med., 65, 661-665 (1995).

Kumar, K.V., Waligora, J.M., Billica, R.D., and Powell, M.R. (abstract) Decompression sickness: Bayesian analysis of individual risk using Doppler-detected microbubbles. Aerospace Medical Association annual meeting, Abstract No. 6, pp A2 (1996).

Van Liew, H.D., Burkard, M.E., and Conkin, J. Testing of hypotheses about altitude decompression sickness by statistical analyses. Undersea Hyperbaric Med., 23, 225-233 (1996).

Low pCO_2 Air-Polarized CO_2 Concentrator Development (Phase I of Space Station Experiment Development Study)

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No Co-Is Assigned to this Task

Funding:

Project Identification: 106-20-01-06
Initial Funding Date: 11/95
FY 1996 Funding: $76,923

Solicitation: 93-OLMSA-07
Expiration: 6/97
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 for space missions requiring low CO_2 partial pressure (pCO_2), i.e., less than one 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 November, 1995, the progress (through September 1996) of the technical tasks has been as follows:

1. Completed review of past work (internal and external).

   Test data for various CO_2 removal systems published in the last five years.

   Flight experiment data published in the International Conference on Environmental Systems (ICES) papers.

   Technology evaluation data/report published by NASA organizations.

2. Identified requirements for critical hardware components of the Electrochemical CO_2 Separator Module (ECSM).

3. Identified ECS electrode (anode) substrate material and its activation sources.

4. Prepared existing hardware, e.g., electrochemical cell modules and Test Support Accessories, for modification.

5. Completed hardware modifications for all Test Support Accessories and for the five cell (approximately one person equivalent) Electrochemical Oxygen Separator Module (EOSM)

6. Completed EOSM testing through checkout, shakedown and design verification testing.

7. Verified that five cell EOSM data was equivalent or exceeded results of previous small scale tests.

8. Readied ECSM for testing.

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.

Space Experiment on Tuber Development & Starch Accumulation for CELSS

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Raymond M. Wheeler; Kennedy Space Center

Funding:

Project Identification: 199-61-17-24
Initial Funding Date: 2/95
FY 1996 Funding: $65,515

Solicitation:
Expiration: 2/97
Students Funded Under Research: 7

Task Description:

Tuber production of white potatoes is of major importance for providing energy-rich carbohydrates in controlled ecological life support systems (CELSS). Starch represents the major source of energy in the edible part of potato tubers, yet existing information implies that accumulation of starch in plant tissues is reduced under microgravity. Thus use of potatoes, and other crops storing large amounts of starch for life support in space, has been questioned, particularly in microgravity of Earth-orbiting stations, but also under reduced gravity on Moon and Mars. This experiment was proposed to study the effect of weightlessness on accumulation of energy-rich starch in potato tubers using excised leaves (explants) on which the axillary buds can be induced to develop small tubers in 10-14 days. This provided an easily controlled system for study of tuber growth and starch production in microgravity on the space shuttle. The experiment was flown in conjunction with the hardware evaluation of the ASTROCULTUR™ plant growing unit developed by the Wisconsin Center for Space Automation and Robotics and included as a middeck locker experiment on the 16 day flight of USML-2 mission in October 1995. The study determined that the tubers formed in the explant system under microgravity had the same gross morphology, the same anatomical configuration of cells and tissues, and the same sizes, shapes, and surface character of starch granules as tubers formed in a 1-G environment. The total accumulation of starch and other energy containing compounds was similar in space flight and ground control tubers. Enzyme activity of starch synthase, starch phosphorylase, and total hydrolase was similar in space flight and ground controls but activity of ADP-glucose pyrophosphorylase was reduced in the space flight tuber tissue. This experiment documented that potatoes will metabolize and accumulate starch as effectively in space flight as on the ground and thus, this data provides the potential for effective utilization of potatoes in life support systems of space bases.

During FY96 effort was concentrated on conducting the experiment on a shuttle flight, undertaking the analysis of the plant tissues generated in space, and summarization of the data for publication.

Plantings of potatoes were made at Hangar L at Kennedy Space Center at two-week intervals beginning in the last two months of FY95 and extending into the first month of FY96. These successive plantings were needed to provide experimental tissue for possible flight delays and for the ground controls. Plantings at two-week intervals were also undertaken in the Biotron at Madison to provide backup plants if necessary for the Kennedy Space Center plants. Explants were harvested from 6-week old potato plants 40 hours before the scheduled flight

time and then loaded into the ASTROCULTURE™ flight package. The first loading was undertaken on September 20 and loadings were repeated four more times because of scrubs and flight delays before the actual launch on October 20. The flight was monitored from the Marshall Space Flight Center, Kennedy Space Center, and the University of Wisconsin by the experimental staff. The downlinking of video images of the explants using the ASTROCULTURE™ camera every other day was of particular value in following the vitality of the explants.

The package was recovered from the Space Shuttle Columbia within 4 hours of touchdown at the Kennedy Space Center and taken to Hanger L for removal of the plant tissue. The explants were photographed, tubers were measured, and then prepared for anatomical and biochemical analysis. Tissue was placed in a formalin based fixative for anatomical studies and frozen in liquid nitrogen for biochemical analysis. The explant harvests were completed within 6 hours of touchdown.

Baseline control studies were conducted in a controlled environment facility (Biotron) at Madison, Wisconsin, one month following the flight. Care was taken to ensure that ground control mother plants, grown at the Kennedy Space Center, were the same age from transplanting as the plants used for the flight and also that the environmental conditions of the middeck during the 16-day flight period were duplicated as closely as possible in the controlled environment room of the Biotron. Explants were harvested from mother plants 12 hours before loading into the ASTROCULTURE™ flight unit and held for this period in small cold storage units over ice. This provided time to carry the explants from Kennedy Space Center to Madison. (The flight explants were also harvested 12 hours before loading into the ASTROCULTURE™ unit and held in the same storage units in a cold room).

Anatomical evaluations were undertaken to compare tubers that developed in space flight to those developing in ground controls. Determinations were made of the size, shape and arrangement of the cells in the different layers of the tubers and measurements made of the cell wall thickness. Determinations were also made of the size and shape of the starch grains that filled the mesophyll cells within the tubers along with study of the size and distribution of protein crystals that were present in these cells.

Biochemical analysis of concentrations of carbohydrates and soluble proteins in the tubers developing both in space flight and on the ground was undertaken. Similar comparisons were made for the activities of enzymes controlling both starch synthesis and starch degradation.

A 90-day report was prepared for the Mission Scientist of the USML-2 flight and reports have been prepared for different scientific meetings and several presentations made before different scientific and local groups. Separate research reports are in preparation on tuber growth, on cellular and subcellular anatomy, and of the biochemistry of the potato tubers developed in space.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Brown, C.S., Tibbitts, T.W., Croxdale, J.G., and Wheeler, R.M. Potato tuber formation and metabolism in the spaceflight environment. SAE Tech Paper 961393. 26th International Conference on Environmental Systems, SAE International, Monterey, CA (1996).

Cook, M.E., Croxdale, J.L., Tibbitts, T.W., Sanwo, M.M., Goins, G.D., Brown, C.S., and Wheeler, R.M. (abstract) Development and growth of potato explants in microgravity. p. 311. 31st COSPAR Assembly, Birmingham, England (14-21 July, 1996).

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 1996 Funding: $ 191,033
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 first aim of this work was to use recombinant engineering to modify the enzyme carbonic anhydrase and to develop a DNA expression system using *E. coli*. Below we detail our accomplishments towards this objective.

We constructed a human carbonic anhydrase type 2 [CA2] expression vector suitable for inserting a six histidine peptide [HIS-tag] and then proceeded to insert the HIS-tag oligonucleotide sequence. CA2 had been cloned and expressed previously by our colleague, Dr. P. Laipis. To insert the HIS-tag, we rearranged the CA2 expression vector by removing a restriction site and inverting the f1 ORI normally used for mutagenesis.

We generated five genetically altered clones and inserted a six histidine oligonucleotide (HIS-tag residue) at either the amino or carboxy termini. Restriction enzyme analysis and DNA sequencing of the resulting clones revealed that four of the five were correct, with a HIS-tag in the correct orientation to allow further use.

Initial assessment of total activity revealed that the amino terminal HIS-tag recombinant enzyme exhibited activity identical with the wild type, unaltered enzyme. While initial carboxy terminus HIS-tagged CA2 clones showed reduced activity (approx. 5-fold), selecting additional colonies from the initial isolates provided several with specific activity comparable to that of the amino terminal-tagged sample. The amino and carboxy terminal-tagged clones were grown as six liter cultures. These showed high total activity and specific activity comparable to the unmodified CA2. Purity of the product is high, >90% on the basis of SDS gel electrophoresis. Both HIS-tag variants showed an appreciable yield (43-48 mg).

Preliminary kinetic studies using 18O indicated considerable similarities in details of the kinetic properties of both the amino and carboxy terminus HIS-tag clones and the wild type enzyme. However, addition of the HIS-tag resulted in some mild perturbations of Kcat and an elevation in activity in the pH titration curve at higher pH as compared with the wild type. While this must be confirmed by more complete kinetic studies, it is clear that the recombinant tagged enzyme is at least as active as wild type CA2.

The second aim was to immobilize the enzyme to a carrier. We tested six different immobilization support surfaces - 3 forms of nylon; methacrylate beads and Ni-NTA sepharose beads. The data revealed that the Ni-NTA provided the most efficient binding - 3-5.5 times more efficient than the other supports.

Given the foregoing data, the focus of the next fiscal year is aim 3, to construct a bioreactor to selectively extract CO_2 from respiratory gases. Our first step is to make and test immobilized enzyme membranes to quantify transmembrane gas capture efficiency.

One possible medical application is development of closed cycle anesthesia machines for gaseous anesthetics. However, carbon dioxide capture followed by concentration or sequestration has many terrestrial applications. Examples of closed environmental life support applications include hazardous materials handling, mine safety, aircraft and submarines, scuba diving, and fire rescue.

A longer term and potentially more significant terrestrial impact of this project will be in scale up of the system as a means of capturing carbon dioxide currently released into the atmosphere, thus helping to ameliorate greenhouse gases. Point sources account for more than one-third of all of the carbon dioxide produced. However, the small size of this system may also allow its use with mobile CO_2 sources. Economically attractive availability of CO_2 is expected to result in development of additional new technologies for applying the available CO_2; examples include enhanced agriculture and aquaculture.

CELSS Crop Simulations for Systems Engineering and Productivity Optimization

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-61-17-23
Initial Funding Date: 9/95
FY 1996 Funding: $0

Solicitation:
Expiration: 8/00
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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. A central objective of this research is to use these experimental findings for (a) systematizing crop data into engineering models that can be integrated into system-level considerations, and (b) analyzing and predicting optimal conditions for new generation of experiments.

The approach will continue the strong collaboration established over previous years with Bruce Bugbee of Utah State University, and also with Ray Wheeler and Gary Stutte of Kennedy Space Center. Benefits to the overall program derive from a modeler working with the experimenters, asking questions, formulating and reformulating models, and publishing collaborative papers that organize the data into a common modeling framework. To address the most important scientific issues about the CELSS crops, the key modeling inputs are the gas exchange data from the above institutions. Gas exchange data are also now becoming available from Ames Research Center and Johnson Space Center.

These general tasks will be specifically accomplished in two major research arenas. First, development of the energy cascade as a modeling strategy that examines the components of crop growth as a sequence of conversion efficiencies. These components require ongoing analysis and prediction because they are relevant to the CELSS Program as fundamental processes of crop growth, and because they are relevant to the CELSS engineers for inclusion in general system design. Second, a new initiative will employ the relatively elaborate field crop models (Ceres-wheat, Soygro, Substor-potato, etc.). Based on the principal investigator's experience with simpler models for the CELSS crops over the years and collaborations with the crop researchers, these field models will be modified and used as modeling tools to predict experiments to increase yield and optimize total life cycle productivity with phasic controls.

Work has progressed on both the Ceres-Wheat model and the Cropgro model for analyzing and predicting NASA experiments with wheat and soybeans, respectively. One of our many modifications to Ceres-Wheat has been to add a two-layer canopy model with specific parameters for direct and diffuse light. The work shows that

characterizing the fraction of diffuse to direct light in experiments with environmental chambers will be significant in determining the reasons for the enhanced yields and perhaps lead to predictions for further enhancements based upon light quality and geometry. We are using the Ceres-Wheat model specifically to explore and predict possibilities for yield improvement during the life cycle with a deliberate schedule of changing environmental conditions, notably temperature. The model indicates that warm conditions early on to speed up vegetative growth, followed by cool conditions later to slow down grain filling would be a regimen for enhanced total grain yield. Moreover, with the model we can explore the effects of switching temperature in all five phases of growth specified by the Ceres-Wheat. In modifying the Cropgro model of soybeans for applications to the experimental growth chamber data of NASA investigators, the key parameters that are proving to be crucial are the leaf expansion rate, the biomass partitioning, and the substrate albedo. As with wheat, it has been proven vital to increase the diffuse component of light in the model in order to explain the high radiation-use efficiency. Results have focused on using experimental data from Utah State University and Kennedy Space Center.

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 on how we have modeled the experimental results from the NASA wheat, we have been able to apply the modified Ceres-Wheat model to explore yield shifts caused by simultaneous warming and higher carbon dioxide levels, both possibilities on a near-future Earth.

FY96 Publications, Presentations, and Other Accomplishments:

Kraft, G., Carr, K.E., Goodwin, E.H., Ting, K.C., Finn, C.K., Tsai, K.C., Volk, T., Henniger, D.L., Mitchell, C.A., MacElroy, R.D., and Stabekis, P. Physical, chemical, biochemical, and biological techniques and processes. Proceedings of the F23.2, F2.3, F4.3, F4.9, F4.10, and F3.5 meetings of the COSPAR Scientific Commission F, Hamburg, Germany, 11-21 July 1994. Advances in Space Research, 18 (1&2), 1996.

Volk, T. Miniaturizing simplified agro-ecosystems for advanced life support. Ecol. Engin., 6, 99-108 (1996).

Performance in Haptic Virtual Environments with Visual Supplement

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Funding:

Project Identification: 199-06-12-40
Solicitation:

Initial Funding Date: 4/95
Expiration: 3/98

FY 1996 Funding: $144,000
Students Funded Under Research: 1

Responsible NASA Center: ARC

Task Description:

The goal of this work is to determine human factors guidelines for effective haptic (force reflecting) manual interfaces for multisensory virtual simulator and teleoperation displays. The two major aspects of this applied research and development project are: 1) the design and implementation of an innovative, high performance three degree-of-freedom (dof) force reflecting manual interface for use with our laboratory's virtual visual display; and 2) examination of human perception and manual task performance, respectively, through psychophysical discrimination and manual target acquisition experiments with the combined haptic-visual virtual environment (VE) research testbed. Application areas for improved force displays include simulator and teleoperator interfaces for design prototyping, training, and maintenance in spacecraft assembly, telescience for planetary exploration, advanced scientific data visualization, and on-orbit physiological and psychophysical research.

During FY96 we completed kinematic and geometric analysis of an innovative mechanical linkage for our three degree-of-freedom (dof) force-reflecting haptic interface. The interface's 10 link, 12 joint "parallel" configuration provides a new fully-backdrivable architecture for coupling 3-D spatial motion to the rotations of three fixed-base electric motor actuators. Prior solutions to the three dof spatial linkage design problem, described primarily in the robotics literature, have relied either on more cumbersome serial linkages, parallel linkages with many more components, or on transmission elements that introduce undesirable friction, backlash, and/or compliance. A U.S. patent application on this new architecture for handcontrollers and robotic manipulators was filed by NASA Ames Research Center in July 1996.

The analysis (Adelstein et al., 1996), indicating two adjacent hemispherical singularity-free volumes bounded by the maximum theoretical extension of the linkage, was followed by the detailed mechanical design, machining, and assembly of a high performance embodiment of this new architecture. As built, the device is scaled to have a 30 cm radius hemisphere of maximum extension. The interference-restricted "achievable" workspace of the device within the hemisphere is comparatively very large: approximately 30 cm wide by 15 cm high by 55 cm deep. This depth corresponds to a range of human arm motion in the mid-sagittal plane that is best suited for stereo vision—i.e., from in front of the nose to full arm extension human arm.

A further innovation of the handcontroller linkage is, contrary to the conventional practice in virtual haptic interface hardware of using aluminum, that it is fabricated principally from stainless steel. The consequence is

higher inertia which serves to mask undesired joint friction and motor cogging, and correspondingly higher stiffness, which also serves to preserve structural bandwidth.

The device's three brushless DC motors, which have skewed magnet stacks to minimize torque ripple, are expected to be capable of maintaining smooth sustained forces of between 20 and 40 N in any direction within the reachable workspace. A six axis sensor joining the endpoint of the three dof linkage to a passive (i.e., non-actuated) three dof spherical handgrip enables full force and torque measurements at the human-machine interface. Precision encoders provide a nominal position resolution of 13 microns at the handgrip necessary for smooth actuator control and for fine detail haptic simulation.

Concurrent to the design and fabrication of the haptic interface, we continued, with shared support from Code UL Task 199061238, to develop advanced real-time software techniques and communication protocols for very low latency (22-27 msec), high update (56-60 Hz) response within the visual component of the virtual environment system hosted by our main laboratory graphics computer. Details of some of these techniques are discussed in the references cited below. These high speed protocols which enable the high degree of temporal fidelity requisite for perceptually stable head-tracked visual display and precise manual interaction will also be employed to integrate the force-reflecting handcontroller into the combined visual-haptic testbed.

The results to this point indicate no significant departures from the proposed research plan.

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.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Adelstein, B.D., Ho, P., and Kazerooni, H. Kinematic design of a three degree of freedom parallel hand controller mechanism. Proceedings, Dynamic Systems and Control, DSC-Vol. 58, American Society of Mechanical Engineers, New York, pp. 539-546 (1996).

Adelstein, B.D., Johnston, E.R., and Ellis, S.R. Dynamic response of electromagnetic spatial displacement trackers. Presence: Telepres. & Virtual Environ., 5(3), 302-318 (1996).

Jacoby, R.H., Adelstein, B.D., and Ellis, S.R. Improved temporal response in virtual environments through system hardware and software reorganization. Proceedings, SPIE Conference on Stereoscopic Displays and Applications VII, Vol. 2653, Bellingham WA, pp. 271-284 (1996).

Kazerooni, H. The human power amplifier technology at the University of California, Berkeley. Proceedings, Dynamic Systems and Control, DSC-Vol. 57, American Society of Mechanical Engineers, New York, pp. 605-613 (1996).

Kazerooni, H. Dynamics and control of instrumented harmonic drives. ASME J. Dyn. Sys., Measur. & Control, 117(1), 15-19 (1996).

Snyder, T.J. and Kazerooni, H. Human force augmentation. Proceedings, IEEE Conference on Robotics and Automation, Minneapolis (1996).

Visual Performance and Fatigue in See-Through Head-Mounted Displays

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Funding:

Project Identification: 199-06-12-38

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 1/98

FY 1996 Funding: $179,000

Students Funded Under Research: 6

Joint Agency Participation: ARPA (TRP)

Responsible NASA Center: ARC

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.

We have used head-mounted see-through displays to examine the cause of errors in human observers' depth judgments to computer generated virtual objects. We have also studied the consequences of monocular, biocular, or stereoscopic viewing on the accuracy of these depth judgments and the subjective viewing discomfort while making them for extended periods of time. The displays used in these studies have been proposed to dramatically increase productivity in several manufacturing environments and to be unique stimulus presentation formats for scientific research. Both of these goals have been advanced by our research. Field trials for some aircraft manufacturing applications are scheduled in mid-1997.

The reduction of measured full system rendering delay for the presentation of head-stabilized stereoscopic virtual objects has, however, remained a significant problem for potential use of these displays. Recent development under this project has reduced the full system delay from 65 msec with 20 Hz updates to approximately 25 msec at near 60 Hz. This performance is currently the best dynamic response of a rendering system like this in the world. These accomplishments have been reported in refereed proceedings papers and as publications in refereed journals. Studies of the accuracy with which monocular systems can display objects in depth using head motion cues have been completed and suggest that further reduction in system latency should significantly improve performance.

The three principle accomplishments of the head-mounted see-through display task in the last year have been: 1) completion of an experiment examining the impact of head motion on the depth rendering of space stabilized virtual objects presented via monocular displays; 2) completion of an experiment examining the trade-off of latency and update rate on 3D human tracking performance with virtual objects and the correlation of this

performance with the subjective reality of the virtual objects; and 3) further reduction of measured full system rendering delay for the presentation of head-stabilized, stereoscopic virtual objects to 22-27 msec at near 60 Hz update rates and initial examination of and implementation of predictive filtering as a means to further reduce the effective full system display lag. These accomplishments have been reported in refereed proceedings papers and as publications in refereed journals.

1) Head motion is shown to improve the judged distance to monocularly viewed, space stabilized virtual objects presented via head-mounted see-through displays. In contrast to similar judgments made with stereoscopic displays, the resulting judgments are not veridical, have considerable variability, and some of their frequency distributions markedly deviate from normality. Comparison of distance judgment to monocularly-viewed real objects suggests that the observer's judgment difficulties arise not from the task itself but imperfections in the displayed imagery. Future work will examine full system display latency as a cause of the imperfect depth rendering observed in our current displays.

2) Many commercial interactive computer graphics systems trade-off throughput, measured in rendered frames/sec versus latency measured as a transport lag. Since these two dynamic properties of interaction have different effects on human performance and since latency may be the more critical, an experiment has been conducted explicitly measuring the human performance trade off of these two for 3-D human pursuit tracking within a virtual object display. Since the two parameters are numeric, they also provide an excellent opportunity to examine the relationship between subject estimates of realism and performance discussed in a review article on the subject in *Presence* (Ellis, 1996).

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: multiple asynchronous UNIX processes communicating via shared memory; continuous streaming rather than polled tracker operation; multiple rather than single tracker instruments; higher bandwidth IEEE-488 parallel communication between tracker and computer replacing RS-232 communication; and hardware synchronization of the spatial tracker instrument with respect to the CRT refresh scan. Average latency of 65 msec and an update rate of 20 Hz for a standard 1000 polygon test VE was improved to near 60 Hz (the maximum achievable with our graphics display hardware) with approximately 27 msec average latency for displacement and 22 msec for orientation. 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 3 have been provided to Boeing Computer Services in response to requests. Code from accomplishment 3 will be made available through appropriate NASA distribution systems if requested.

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 the latter site, head-mounted displays for wearable computers have been shown to dramatically increase productivity of workers examining KC135 fuselages for cracks in their skin.

Virtual environment displays can be used to present visual, acoustic or haptic stimuli used in psychological or physiological investigations and thus can help advance scientific research. In fact, the virtual display format

makes possible the presentation of patterns of sensory information that are not physically realizable and can give researchers 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 3-D data visualization, geographic information systems, entertainment, and video games. More detailed discussion of the widespread applications of virtual environments can be found in the general reference articles cited below.

FY96 Publications, Presentations, and Other Accomplishments:

Adelstein, B.D., Johnston, E.R., and Ellis, S.R. Dynamic response of electromagnetic spatial displacement trackers. Presence, 5(3), 302-318 (1996).

Ellis, S.R. Presence of mind: A reaction to Sheridan's musings on telepresence. Prescence, 5(2), 247-259 (1996).

Ellis, S.R. Virtual environments and real imagination. Current Directions in Psychological Science (in press).

Ellis, S.R. and Menges, B.M. Judged distance to virtual objects in the near visual field. Presence (in press).

Ellis, S.R. and Menges, B.M. Effects of age on judged distance to virtual objects in the near visual field. Proceedings of the Human Factors and Ergonomics Society, 40th Annual Meeting. Philadelphia, PA, pp. 1197-1201 (1996).

Ellis, S.R. and Menges, B.M. Spatial interaction of virtual objects and physical surfaces seen through head-mounted displays. Psychonomics Society Meeting, Los Angeles, CA (November 10, 1995).

Jacoby, R.H., Adelstein, B.D., and Ellis, S.R. Improved temporal response in virtual environments through system hardware and software reorganization. Proceedings of the SPIE, Stereoscopic Displays and Virtual Reality Systems III, vol. 2653, pp. 271-284 (1996).

Noor, A.K. and Ellis, S.R. Engineering in a virtual environment. Aerospace America, July, 32-37 (1996).

Perceptually-Tuned Visual Simulation

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Project Identification: 199-06-12-05
Solicitation: 93-OLMSA-07
Initial Funding Date: 5/95
Expiration: 4/98
FY 1996 Funding: $158,000
Students Funded Under Research: 5

Responsible NASA Center: ARC

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 three-dimensions based upon principles of visual motion processing. This research requires a multidisciplinary approach and will involve a collaboration among research scientists at the NASA Ames Research Center, professors in Computer Science and Psychology/Biomedical Engineering at the University of Virginia, and designers and engineers at Silicon Graphics, Inc. and other industry sites.

During FY96, we made a number of significant accomplishments and developments. User evaluation studies were conducted to examine the efficacy of high-low stereo displays (and test for any undesirable artifacts or aftereffects). The findings of these studies are quite promising: participants' ability to extract stereo-specified depth information from high-low display was almost equivalent to that with conventional (high-high) displays; there was no discernible impact on the participants' stereo vision, as assessed by pre- and post- stereoacuity tests. These findings were presented at SIGGRAPH'96.

Several of the researchers supported by this grant supported the Mars Virtual Exploration Control Center Exhibit, mounted at the National Air and Space Museum as part of the Viking 20th Anniversary Celebration.

This exhibit permitted real-time interaction with the Mars Digital Terrain Database on a mid-size graphics workstation (a major advancement over earlier visualization efforts for this data set). Issues associated with such real-time visualizations, including how they can be aided by perceptually tuned rendering techniques, are detailed in an article currently in press in the journal, *Behavior Research Methods, Instruments, & Computers* (and will be included in next year's update).

The dissemination of these techniques was facilitated by paper presentations at SIGGRAPH, American Psychological Society, and the Society for Computers in Psychology, as well as symposia and discussions at several organizations including Interval, Silicon Graphics, and Altantis Corporation. The patent for the variable resolution rendering techniques is still pending; NASA is initiating an Interference Process with the U.S. Patent office to resolve the issue of a competing patent.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Hecht, H., Kaiser, M.K., and Banks, M.S. Gravitational acceleration as a cue for absolute size and distance. Perception & Psychophysics, 58, 1066-1875 (1996).

Kaiser, M.K. High power graphic computers for visual simulation: A real-time rendering revolution. Behavior Res Methods, Instruments & Computers, 28, 233-238 (1996).

Kaiser, M.K. Downsizing visualization platforms: From Crays to Indigos and beyond. Symposium on High-Performance Computer Applications in the Behavioral Sciences, (May 1996).

Kaiser, M.K. Perceptually tuned visual stimuli. American Psychological Society 8th Annual Convention (June 1996).

Proffitt, D.R. and Kaiser, M.K. "Perception of space and motion" in "Handbook of perception and cognition." Academic Press/Orlando, FL, 5, pp 227-261, 1995.

Proffitt, D.R. and Kaiser, M.K. Hi-Lo stereo fusion. SIGGRAPH '96 (August 1996).

An EVA Strength and Reach Model

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Funding:

Project Identification: 199-06-11-46
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/97
FY 1996 Funding: $60,000
Students Funded Under Research: 2

Responsible NASA Center: JSC

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 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. Motion analysis data along with collected multi-joint motion torque data will be used to validate the EMU kinematic and strength model.

Data Collection:

The strength limitations of the EMU-suited crew member are not well understood. There are severe strength and reach limitations imposed by the EMU suit. No comprehensive strength data exists for the isolated joints of an EMU-suited astronaut compared with his unsuited strength. During this year, isolated joint strength data in both the suited and the unsuited condition has been collected and processed. The Flight Crew Support Division's Anthropometry and Biomechanics Facility (ABF) and the Graphics Research and Analysis Facility (GRAF), in cooperation with the Crew and Thermal Systems Division (CTSD), have completed a study that collected and

processed quantitative data for the isolated joint strength capability of six suited operators. A generic methodology for quantifying the differences between and within the unsuited and different suited conditions has also been established.

Modeling:

The EMU model has been revised for more accurate modeling and to include the new enhanced suit design. The goals of the project are to develop an accurate EMU model by incorporating much of the sizing algorithms developed by the EMU Suit Facility (ESF). The ESF uses an elaborate scheme to fit an actual astronaut into an EMU. The aim of this project is to attempt to programmatically encapsulate this scheme. First, all new component models were constructed to more closely resemble the pieces of the Enhanced EMU. Next, development of the EMU Sizing Program (ESP) was completed. ESP reads in anthropometric and sizing criteria data to build an EMU model composed of the new components to fit the subject. ESP simulates the process performed by an engineer in the ESF fitting the EMU to an actual astronaut. ESP returns a command file to be run in the Dynamic Manipulator Composite Object Generator software which outputs the actual components of the Enhanced EMU model as well as the (Intravehicular Activity) IVA human model.

Inverse Dynamic Strength Model Formulation:

The center of mass and moment of inertia data were gathered for various components of the human model. Equations of motion were generated for a simple test case using a software program called SD/FAST. This program provides low level numerical integration routines which are being used to produce a dynamic simulation of a linked system. The ultimate goal of this approach is to be able to predict EMU mass handling properties through an inverse dynamics computation and compare the required torque values with collected data on the EMU.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Pandya, A., Hancock, L., Morgan, D., and Maida, J. (poster) Analysis of human posture using a strength model and a virtual environment. 14th Annual Houston Conference on Biomedical Engineering Research (Feb. 1996).

Human Task Performance Evaluation with Luminance Images

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-06-11-55
Solicitation: 95-OLMSA-01
Initial Funding Date: 2/96
Expiration: 2/97
FY 1996 Funding: $100,000
Students Funded Under Research: 1

Responsible NASA Center: JSC

Task Description:

This Space Human Factors proposal addresses a need to develop high-fidelity mockup and training simulators with the goal of providing cost-effective technology for facile evaluation of dynamically changing mission scenarios and training for operations in the harsh and rapidly changing lighting conditions of space.

NASA currently relies on ground tests to evaluate the influences of harsh on-orbit lighting conditions on Space Shuttle mission activities. These ground tests are expensive to carry out and must be repeated as mission parameters change in the course of planning. A more flexible and less expensive means to accomplish the same ends uses computer image computations of the lighting conditions in a space environment. The Graphics Analysis and Research Facility (GRAF) has demonstrated a predictive capability to construct maps of light intensity, called luminance maps, that agree with ground-test and on-orbit data. GRAF proposes to extend these luminance maps into simulated camera images that recreate solar-lit or artificially-lit on-orbit scenes as viewed by on-board cameras and evaluate whether these luminance images, when applied in a computer-based mission evaluation and training simulator, provide experience to the crew in handling the rapidly changing lighting conditions which will enhance their performance of tasks in space.

There are two stages of research necessary to provide this proposed capability, prior to application in a more complex training and mission evaluation environment. First, a software program to convert the luminance maps into simulated camera images, to provide human interpretable TV pictures, needs to be developed and validated. Second, using a simple remote operater manipulation task, the degree of correlation of training with simulated camera images to task performance enhancement for mission operations needs to be determined. GRAF proposes to perform both stages of this research.

Project Objective

A goal of this project is to be able to generate computer images from luminance maps which will accurately simulate specific camera images. These images must be able to change according to the camera parameters which are used and the type of camera selected. Another goal is to test the use of luminance images as an improvement to training scenarios where lighting can be a factor in performance.

Development of a Camera Model

The CTVC has two major settings which impact the image, Automatic Light Control (ALC) and Gamma Correction Control. These settings can now be modeled synthetically. These models can be use as performance predictions under specific illumination conditions. Preliminary studies have found their predictability to be extremely useful for flight planning of the Canadian Space Vision System on STS-80.

Validation of the Camera Model

Currently, crew training and familiarization of the onboard camera system (part of the rationale for this project) involves the use of a Mir docking module and target mockup, a solar simulator and flight-like cameras with camera control. These sessions are conducted in a darkened environment. Camera images, camera settings, and the associated luminance and illuminance measurements of major surface areas of the docking module were collected. Results are currently being examined on a case-by-case basis.

Validation of Camera Model with actual STS-80 flight data was also performed. Predictive models about shadow and glare locations were generated in July of 1996. These images were then compared to downlinked images from STS-80 during its mission in November of 1996. Predictions were extremely close even though the launch date had slipped several days.

Testing the Effects on Training

In order to quantify the effects of illumination on training, a test stand has been developed. The testing hardware consists of a small docking module mockup illuminated by a small solar simulator, much like the current crew training described but on a smaller scale. This will provide the basis for comparing computer simulated images of the same scene using computer modeled illumination. Initial testing and adjustments are underway. The subjects will be "flying" the orbiter toward an aligned position and will be graded on several parameters to determine performance.

Because the modeling of illumination for use with training simulators may have an important effect on training in general, the benefits could be considered very general as well. For instance, tasks which may be performed during night hours such as operating a large ship, a truck, or an aircraft will be affected by visibility and less than perfect lighting conditions. Even a mundane operation such as driving an automobile will be affected. If training measures are identified which can make an operator more sensitive to these restrictions, the safer and smarter the operator will become.

FY96 Publications, Presentations, and Other Accomplishments:

Maida, J.C. Measurement and validation of bidirectional reflectance of space shuttle and space station materials for computerized lighting models. NASA Tech. Brief, NASA Tech. Paper 3649.

Human Interaction Design for Cooperating Automation

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Funding:

Project Identification: 199-06-11-50
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $108,000
Students Funded Under Research: 1

Responsible NASA Center: JSC

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 FY96, the case studies focused on intelligent situation capture and review for space shuttle flight controllers from the Mechanical Maintenance and Crew Systems (MMACS) discipline. The human interaction design storyboards and requirements are complete for the payload bay door (PLBD) case. The software captures and organizes real-time data associated with PLBD operations events and provides situation review displays. The review displays use an organized log presentation to identify anomalies and aid comparison of expected with observed operations, durations, and data. Human interaction design and the first prototype are also complete for SPORT (Situation Playback Orbiter Data Reduction Complex (ODRC) Retrieval Tool), which provides real-time data playback for situation review and automates data-retrieval requests.

A draft Human Interaction Design Field Guide Methods Description was completed and made available on the World Wide Web. This guide to methods and process will be revised next year to include lessons learned in the case studies and methods developed in the project. Design products from the case studies have been linked to the Field Guide.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Malin, J.T. (abstract) Intelligent systems as team players. Minutes of the Department of Defense Human Factors Engineering Technical Advisory Group, 36th Meeting, Houston, TX: Abstract P-1 (May 1996).

Malin, J.T. Cooperating automation: Event-oriented situation displays. Minutes of the Department of Defense Human Factors Engineering Technical Advisory Group, 36th Meeting, Houston, TX: Abstract Q-2 (May 1996).

Malin, J.T., Thronesbery, C.G., and Schreckenghost, D.L. (abstract) Progress in human-centered automation: Communicating situation information. Life Sciences and Space Medicine Conference: A Book of Abstracts, AIAA, Houston, TX: 88-89 (March 1996).

Woods, D.D., Patterson, E.S., Corban, J.M., and Watts, J.C. Bridging the gap between user-centered intentions and actual design practice. Proc. Human Factors and Ergonomics Soc. 40th Annual Meeting, Philadelphia, PA (September 1996).

Perceptual Optimization of Image Compression and Displays

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Project Identification: 199-06-12-39
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $266,000
Students Funded Under Research: 5

Responsible NASA Center: ARC

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 FY96 has been the completion of a study applying the DCTune perceptual optimization technology to x-ray medical images. These experiments were designed to answer three questions: 1) Does the DCTune image quality metric provide meaningful estimates of image quality? 2) Does the DCTune optimization technique provide better image quality the prior art? and 3) Is it possible to design quantization parameters that are optimized for a class of images, rather than a single image? All three questions were answered in the affirmative.

We have extended the DCTune perceptual optimization technique to the case of spatially adaptive quantization. We have shown that DCTune can produce considerable increments in the quality of adaptively coded still images, such as in the JPEG extension. This is an important precursor to perceptual optimization of MPEG, which uses adaptive quantization.

We have continued our 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. 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 Dartmouth College. We will also commence work on perceptual compression of moving images (video) in the context of the MPEG algorithm.

Another important focus of the work in FY96 has been to understand better the process of visual masking, particularly as it applies to the problem of perceptual optimization of image compression. In this context we have developed the method of classification images, which directly reveal the observers weighting of image features in the detection process. We have also conducted experiments that reveal a phenomenon we call entropy masking. This is the masking induced by an unknown background, and arises from the inability of the observer to discount or ignore the background.

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.

In support of our psychophysical work, we have continued to develop software tools for easy generation of calibrated displays and experimental protocols on personal computers. Two papers describing these contributions have been published.

During the past year, the PI has served on several program committees for major research organizations, has chaired several conference sessions, and delivered three invited or keynote talks. He was elected chair of the Vision Section of the Optical Society of America. His compression technology has received inquiries from at least fifteen companies to date.

The Earth benefits of this research will be manifest in any enterprise that relies on visual communication of information. Significant examples are medical imaging, Earth resource imaging, space imaging, science imaging, and internet imaging. In each case there is a need for efficient archiving and distribution of digital images, and high quality display of those images. Advances in medical imaging in particular may be expected to enhance diagnostic capabilities and to reduce costs of medical care. Earth resource imaging may be expected to reduce environmental damage and reduce costs of detection and repair of such damage.

In a more general sense, visual displays are at the heart of the modern technology revolution, from laptop computers and the world-wide-web to high-definition television, virtual reality, and telepresence. Improvements in the efficiency and quality of visual imaging and displays will have ramifications throughout our technological infrastructure and economy. Beyond its technological payoff, the basic component of this research promises new understanding of the fundamental mechanisms of human vision, especially in the areas of visual detection and motion perception. This understanding will assist in analyzing visual diseases and injuries, and in developing appropriate therapies.

FY96 Publications, Presentations, and Other Accomplishments:

Ahumada, A.J., Jr. "Simplified vision models for image quality assessment" in "Society for Information Display International Symposium Digest of Technical Papers." Edited by: Morreale, J. Society for Information Display, Santa Ana, CA, (1996).

Ahumada, A.J., Jr. (abstract) Perceptual classification images from Vernier acuity masked by noise. Perception 26 (ECVP Supplement), 18 (1996).

Ahumada, A.J., Jr. and Beard, B.L. "Object detection in a noisy scene" in "Human Vision, Visual Processing, and Digital Display VII." Edited by: Allebach, B. R. SPIE, Bellingham, WA, Paper 23, (1996).

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Mulligan, J.B. (abstract) Eye-movement tracking using fundus images. Optics and Photonics News, 7(8) Supplement, 82 (1996).

Mulligan, J.B. Image processing for improved eye tracking accuracy. Beh. Res. Meth. Instrum. & Comp., (in press).

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Human Interaction Design for Anomaly Response Support

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Project Identification: 199-06-17-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $63,000
Students Funded Under Research: 1

Task Description:

We have conducted a cognitive analysis of anomaly response which focused on the coordination occurring across a complex system of interdependent teams in space shuttle mission control. The data includes results from observations, interviews, and reviews of past anomaly cases. Our analysis activities have focused on the distributed nature of anomaly response in the space shuttle mission control domain, and have allowed us to develop a framework for our cognitive model, as well as ideas for aiding concepts to support anomaly response.

We have combined the information gained from our research and analyses with principles of effective human computer interaction to produce design concepts for useful tools to support distributed anomaly response. These design concepts will allow NASA to develop effective anomaly response support tools as the mission control structure changes to adapt to shrinking resources. We are currently developing prototypes to demonstrate our design concepts.

To date, products include a general model of the cognitive processes involved in distributed anomaly response and a detailed description of anomaly response in the space shuttle mission control domain. We have also finished one prototype of an anomaly response support system. We are currently working on a second iteration of prototypes that will allow us to further explore aiding concepts for supporting anomaly response, as well as generic design concepts for supporting the development of anomaly response support across disciplines.

To date, we have conducted observations, interviews, and reviews of past anomaly cases. 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 (now called the MOIR) to further investigate the activities necessary for anomaly response. These activities have focused on the distributed nature of anomaly response in the space shuttle mission control domain, and have allowed us to develop our cognitive model of anomaly response, as well as ideas for aiding concepts to support the anomaly response process. We have also created an initial prototype to explore some of these aiding concepts.

In addition, we have completed our general model of anomaly response and our detailed description of anomaly response in the space shuttle mission control domain. Papers include 1) exploring how voiceloops support coordination presented at the Computer Supported Cooperative Work (CSCW) conference in November 1996 and

2) a dissertation entitled "A cognitive analysis of functionally distributed anomaly response in space shuttle mission control," submitted to Ohio State University in December 1996.

Currently, we are working with shuttle mission control practitioners to develop a second generation of prototypes that have evolved from our cognitive model. Our model of anomaly response will grow and evolve as we continue to develop and evaluate prototypes based on our aiding concept ideas.

This research will benefit society in general by further developing our understanding of how to create tools to effectively support human problem solving. For example, by studying anomaly response in a distributed domain like space shuttle mission control, we are learning how to develop tools that effectively support group coordination and distributed problem solving. Our results should be applicable to many domains where problem solving is distributed across groups of people.

FY96 Publications, Presentations, and Other Accomplishments:

Watts, J.C., Woods, D.D., and Patterson, E.S. Functionally distributed coordination during anomaly response in space shuttle mission control. Proceedings for the 3rd Annual Symposium on Human Interaction with Complex Systems (HICS '96), Dayton, OH (August 1996).

Behavioral Trends and Adaptation During Space Analogue Missions

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Funding:

Project Identification: 199-16-11-14
Solicitation: NRA-93-OLMSA-07
Initial Funding Date: 10/94
Expiration: 9/97
FY 1996 Funding: $ 100,000
Students Funded Under Research: 0

Responsible NASA Center: JSC

Task Description:

The proposed investigation is the first in a series of behavioral science studies designed to examine different aspects of psychological adaptation during long-duration missions, and in other isolated, confined environments. This investigation has two objectives: 1) identify and characterize trends in psychological and behavior variables over the course of long-duration analogue missions; and 2) obtain data to support the development of more specific hypotheses regarding psychological and behavioral changes in long-duration missions.

Many incidents reported during space missions flown by the United States and Russia have been attributed to reports of friction among crew members and lapses in judgment. A number of factors, such as isolation and confinement, are presumed to account for behavioral problems that occur in space. In order to understand and prevent undesirable changes, we must first ascertain the events and conditions that cause or influence these changes. Second, we need to measure the impact of behavioral and psychological changes in terms of health and performance readiness. Finally, we need to examine how individuals deal with behavioral and psychological changes when they occur. This descriptive study will use a pooled time-series approach to collecting and analyzing self-report measures of psychological and behavioral variables throughout.

Data collection has been completed on four year-long Australian Antarctic stations, with 8-12 respondents at each site. These data have been partially analyzed and will be presented at several upcoming scientific meetings. Additional data were collected during the FY96 Austral winter in these same stations. These data are being reduced and formatted for analysis. Preparations for FY97 winter data collection were completed, and data collection is currently in progress. In addition, we completed data collection and have partially analyzed data from 4 crew members who participated in Phase II of the Early Human Testing Initiative (EHTI) at JSC for 30 days.

Although data collection and analysis is ongoing, some preliminary observations include the following:

1) Results from time series regressions suggest that personal factors of the individual crew members, and local events are the primary causes for changes in psychological factors in both Antarctic and EHTI crews;
2) Interpersonal tensions increase almost linearly throughout the period of isolation; and
3) Relationships with remote management decline throughout the period of isolation.

In both environments, crew members successfully completed their assigned tasks and met all mission objectives. Differences between the two groups may be due to differences in crew selection processes, group history, degree of isolation, degree of visibility during isolation, and length of isolation.

This study uses a pooled time-series approach to collecting and analyzing self-report measures of psychological and behavioral variables throughout the period of isolation. Our preliminary results indicate that this approach has the potential to identify the events and personal characteristics that affect individual psychological adaptation and group functioning in order to answer critical questions concerning selecting and composing teams (crews) for long-duration space missions.

This year's progress has led us to conclude that by combining data across groups and different environments, we can begin to triangulate on the potential problems that groups in space will encounter and to evaluate coping strategies. We recommend that future work in this area include comparisons of different environments, and research directed toward selecting and composing teams for long-duration isolated environments.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Wood, J., Harm, D.L. and Eksuzian, D.J. Psychological considerations for humans in bioregenerative life support systems. Presented at 31st COSPAR Scientific Assembly, Birmingham, UK, July 14-21, 1996.

Crew Culture, Selection, Training and Performance

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Funding:

Project Identification: 199-06-17-03
Initial Funding Date: 10/94
FY 1996 Funding: $

Solicitation: 93-OLMSA-07
Expiration: 9/95
Students Funded Under Research: 10

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

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.

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Crew Behavior and Performance in Ground Operations

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Project Identification: 199-06-12-04

Solicitation: 93-OLMSA-07

Initial Funding Date: 5/95

Expiration: 4/96

FY 1996 Funding: $0

Students Funded Under Research: 2

Joint Agency Participation: DoD

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Responsible NASA Center: ARC

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 two 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.

During FY96, we completed two studies in which process analysis techniques were explored. The first study used field observational methods for analyzing how delays and interruptions occur in the shuttle processing domain. The second study analyzed human error incidents in the aircraft maintenance domain by utilizing a large incident database from the NASA Aviation Safety Reporting System. In April 1996, a panel consisting of both

Ames Research Center and Kennedy Space Center participants discussed "Team Effectiveness in the Space Launch Environment" at the 15th Symposium of the Applied Behaviors Sciences sponsored by the USAF Academy Department of Behavioral Sciences and Leadership.

Because the analysis of human error incidents is an issue shared by both aircraft and space ground operations, ARC and KSC organized a workshop in which such issues could be discussed and "lessons learned" could be shared. This workshop, entitled The Analysis of Errors (incidents, mishaps, and close-calls) in Aerospace and Aircraft Maintenance Domains, took place in September, 1996 and was the first in a planned series.

We have also investigated the ways in which human factors training might be shared across aircraft and space maintenance domains. A second NASA ARC - KSC Human Factors Workshop will focus on this topic in May, 1997.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Irwin, C.M. and Kanki, B.G. (Poster presentation) The nature of procedural interrupts in ground operations in aerospace systems. Annual Meeting of Human Factors and Ergonomics Society, San Diego (1995).

Kanki, B.G. Crew resource management and technical operations. The CRM Advocate (of Resource Options, Inc.), 96 (2), (April 1996).

Kanki, B.G. The analysis of errors (incidents, mishaps and close-calls) in aerospace and aircraft maintenance domains. Proceedings of the NASA Ames Research Center - Kennedy Space Center Human Factors Workshop I, Moffett Field, CA: NASA Ames Research Center (September 1996).

Kanki, B.G. (abstract) Team effectiveness in the space launch environment: Theory to applications. 15th Symposium of the Applied Behaviors Sciences sponsored by the USAF Academy Department of Behavioral Sciences and Leadership, Colorado Springs, CO (April 1996).

Veinott, E.S. and Kanki, B.G. (Poster presentation) Identifying human factors issues in aircraft maintenance operations. Annual Meeting of Human Factors and Ergonomics Society, San Diego (1995).

Development of Data-Driven Models to Describe Astronaut Performance in Microgravity: Full-Body Dynamics and Control

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Funding:

Project Identification: 199-70-17-21
Solicitation: 93-OLMSA-07
Initial Funding Date: 1/95
Expiration: 1/98
FY 1996 Funding: $82,235
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 zero-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.

Computational multi-body dynamics were used to simulate astronaut extravehicular activity (EVA) tasks. Two actual EVAs were simulated: manipulation of the Spartan astrophysics payload on STS-63 and attempts at capturing a spinning Intelsat VI satellite on STS-49. This research effort fills a current gap in quantitative analysis of EVA by employing computational dynamics, with emphasis on Kane's method, to solve the equations of motion for the dynamics of the astronaut's body segments and other interacting objects. The simulation approach can be divided into six phases: (1) model design, (2) system description, (3) equation formulation, (4) inverse kinematics, (5) inverse dynamics, and (6) data display with animation. The Spartan simulation is performed using a relatively simple seven segment astronaut body model with six degrees of freedom and motion restricted to a single plane. Results of the Spartan simulation reveal how an analyst might predict difficulties imposed by task specifications requiring violation of physiological limits, and modify the protocol so that the tasks objectives are humanly achievable. The more complex Intelsat simulation, using a 12 segment astronaut body model with 31 degrees of freedom, and interacting capture bar and satellite objects, each with 6 degrees of freedom, reveals greater challenges in terms of motion control and numerical integration. Interaction between the capture bar and satellite is modeled by means of constraint forces imposed at two contact points and achieves realistic motion of the two objects. Collision between the capture bar and Intelsat produces high acceleration spikes, which when used to perform prescribed motion of the astronaut body model lead to instabilities in the motion integration and high joint torque values. An initial attempt at controlling these instabilities produced improved transient behavior, but was unable to avoid eventual divergence. Future approaches to this problem, such as Baumgarte stabilization, are suggested. Another contribution of this research effort was to develop a dynamic model of the extravehicular mobility unit (EMU), or current NASA space suit. The EMU model incorporates three key suit parameters, namely, mass, inertia and performance for EMU components including the portable life support system (PLSS). Replicating the Spartan EVA simulations while including the space suit model reveals that the astronaut does nearly an order of magnitude more work to produce the same results when the initial conditions are such that the lower body is fixed. Allowing for a compliant lower body astronaut model with the suit model results in the suited and unsuited condition requiring similar amounts of work by the astronaut. An interesting result is that the initial conditions from which the astronaut starts the task greatly affects the results (i.e., astronaut neutral body posture versus EMU neutral suit posture in microgravity). Finally, a series of simulations was performed to assess the effect of a space suit on an astronaut engaged in repetitive motions over a long time representative of future International Space Station (ISS) tasks. The accumulation of additional work to overcome space suit properties might lead to accelerated muscle fatigue during these simulations.

The new issues being addressed are the application of the new suit model to appropriate EVA tasks, such as station construction EVAs; the implementation of a musculoskeletal model for physiological accuracy; the incorporation of space flight data into muscle and control models; and the integration of the EVA model into pre-existing software at NASA facilities.

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.

The EMU space suit model is easily extensible to confined-environment and suited activities on Earth, such as research and industrial diving, Antarctic investigation, and other extreme-environment tasks involving the encumberment of an exposure suit.

At the basic biological level, this research effort has progressed in applying engineering adaptive control theory to model the central nervous system (CNS) and lower level involvement in the maintenance of posture. Again, these techniques provide a more rigorous analytical method for clinical use.

The analysis 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.

FY96 Publications, Presentations, and Other Accomplishments:

Newman, D.J. Engineering analysis of astronaut adaptation in altered gravity. University of Colorado at Boulder, Department of Aerospace Engineering, Boulder, Colorado (September 9, 1996).

Newman, D.J. EVA human performance in reduced gravity. International Space University, Department of Space Systems and Mission Design, Technical University (TU) in Vienna, Austria (August 13, 1996).

Newman, D.J. Modeling the human operator in microgravity. Department of Aeronautics and Astronautics Century Symposium, Massachusetts Institute of Technology, Cambridge, Massachusetts (June 13, 1996).

Newman, D.J. Simulation, modeling, and virtual reality for planetary mission planning. International Space University, Department of Space Systems and Mission Design, Technical University (TU) in Vienna, Austria (August 14,1996).

Newman, D.J., Schultz, K.U., and Rochlis, J.L. Closed loop, estimator based model of human posture following reduced gravity exposure. J. Guidance, Control & Dyn., 19(5), 1102-1108 (1996).

Distributed Decision Making in Extended Space Flight

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Funding:

Project Identification: 199-06-12-36
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $340,000
Students Funded Under Research: 5
Joint Agency Participation: FAA, DoD

Responsible NASA Center: ARC

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: team structure and expertise, cultural variability, communication medium, 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 and performance.

1. **Team Communication and Decision Processes.** In our earlier work on Team Communication and Decision Making, we developed a process model to characterize how teams make decisions in dynamic complex environments. As a result of recent experiments, we modified the model to reflect the importance of time pressure and risk in the situation assessment component. While time pressure constrains information gathering and exploration of alternatives, it is important for crews to assess the actual amount of time available for making a decision. Even experienced individuals sometimes behave as though time is limited when it is not, resulting in poor decisions because of failure to consider all available information. This is especially common under stressful conditions. Accurate risk assessment is also critical to good decision making, especially when the situation is ambiguous and is changing dynamically. Apparent underestimation of the severity of risk has been associated with many accidents involving human judgment. The concept of risk has been expanded to include threat of legal violation; conflict with organizational policy, values, or goals; social threat; and ethical issues, in addition to danger. These various types of risk may operate differently on decision processes. Efforts to support effective decision making should target decisions that are most difficult or likely to result in errors. Responses from pilots indicate that difficult decisions involve ambiguity, goal conflict, high risk (danger), and absence of guidance or no good solution available. Strategies that were associated with effective decision

making by pilots in full-mission simulators included (a) managing their time and resources so they could devote their attention to solving the problem; (b) using all available resources, including team members on the ground, to assist either strategically or with information or options; and (c) taking into account more problem-relevant information. Future work will examine alternative perspectives on risk and risk management strategies.

2. **Cultural Diversity and Crew Communication.** 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. Studies are underway to address cultural differences in the language used by crew members to call attention to a problem or to an error committed by the other crew member, who may be of a higher or lower status. Flight-related scenarios have been developed that differ in the degree to which "face" is challenged by calling attention to the problem and that differ in level of risk. An initial study conducted with pilots from two major U.S. airlines indicated that captains are more direct in addressing first officers than are first officers in addressing captains. Moreover, communication is less mitigated in high-risk situations than in low-risk situations. Studies will be done with pilots from a variety of English-speaking and non-English speaking countries that differ from the U.S. in power distance and group orientation to determine effective communication strategies. We are also addressing the effect of gender of the speaker and hypothetical addressee with U.S. pilots. A second line of work is examining types and sources of miscommunication between flight crews and air traffic controllers in parts of the world where participants do not speak the same native language. Incident reports of miscommunications involving culture and language have been classified into types of errors; the causes and consequences of communication errors between flight crews and controllers are being analyzed.

3. Remote Diagnosis for Trauma Patient Resuscitation. This study addresses the cognitive demands of distributed medical decision making as it pertains to the treatment of acute trauma patients. In the first phase of the study, six anesthesiologists experienced in trauma patient care attempted a remote diagnosis task. They were presented with audio-video case segments of trauma patient resuscitation and were asked to report their understanding of patient and resuscitation status. Major findings of the analysis include: (1) critical visual and auditory cues were often missed by the subjects; (2) the subjects seemed to be overloaded by multiple activity threads contained in the audio-video scene; (3) patient history information was critical for the subjects to understand audio-video scenes; and (4) secondary cues (such as facial expressions visible from the video scenes) were used to determine patient resuscitation status. In a second study, four attending trauma surgeons and four experience trauma nurses performed the same remote diagnosis task. Findings from the two studies indicated that the three groups of experts (surgeons, nurses, and anesthesiologists) differed in their abilities to detect critical cues and offer diagnostic suggestions. It appears that experts were bounded by their specialized roles in a resuscitative team. For example, the anesthesiologists were able to understand airway management related patient and resuscitation status better than the surgeon and nurse subjects. These findings suggest that to provide effective consultation in dynamic, multidisciplinary team activities, either an assembly of teleconsultants or special training are needed. Detailed data analysis is ongoing to contrast the strategies used by the three expert groups. A new remote diagnosis task has been prepared in which the subject will be given audio-track of resuscitation activities with verbal narrative comments. Further experiments are planned to use this task to determine the value of the video medium.

4. Effects of Prolonged Isolation on Team Decision Making. Little systematic evidence exists on how prolonged isolation affects team performance on types of tasks that will be especially critical for space missions—challenging, complex tasks that require team coordination for success. This study is examining the effects of isolation and confinement on team decision making in Antarctic overwintering personnel. Behaviors that may be sensitive to isolation effects include risk taking, cooperation and competition, resource and information sharing, dynamic workload distribution, and team coordination. A Distributed Dynamic Decisionmaking tool (DDD-III) has been developed and will be used to collect data from overwintering teams. Scenarios are being developed that will prove challenging and interesting to these teams, as they will be tested repeatedly over the course of the winter. Data will be collected from non-isolated US teams as a baseline for comparison with the isolated teams. Progress is well under way on porting the DDD system to a PC environment.

Earth benefits from this project are expected in three areas: training, design of procedures to enhance team decision making, and specification of requirements for decision aids. Training will apply both to individuals and teams that operate in dynamic high-risk environments. The decision process model developed under this grant is most directly applicable in the aviation domain, where pilots, air traffic controllers, dispatchers, and maintenance specialists often must pool their resources to solve problems. To date, several airlines have adopted our dynamic decision process model as a framework for training their flight crews to assure greater safety within the aviation system. Findings are also being applied in other industries where technical specialists and managers must cope with problems and make decisions, such as management of off-shore oil platforms, nuclear power operations, and fire fighting.

The analysis of cultural and gender factors in effective team communication will be relevant for designing procedures to facilitate and support communication in multicultural settings in many technical domains, including air traffic management. Likewise, results of the effects of stress identified in overwintering Antarctic teams' performance may provide insights to support other Earth-based teams operating in high-stress environments.

Findings from our remote medical diagnosis effort will be directly applicable to telemedicine on Earth, where medical practitioners cannot observe patients first hand due to their remote location or where a distant specialist may be required. This project will yield information about what kind of information and structure of inquiry are most useful for remote diagnosis, tailored to the level of knowledge and expertise of the practitioner.

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Review and analysis of Diaries from French Remote Duty Stations

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Project Identification: 199-08-17-75
Initial Funding Date: 7/96
FY 1996 Funding: $50,676

Solicitation: 95-OLMSA-01
Expiration: 6/97
Students Funded Under Research: 0

Task Description:

This research project involves the translation and analysis of personal journals that were maintained for this purpose by the station leaders and medical officers of the Dumont d'Urville Antarctic facility and other French remote duty stations located on small islands in the South Indian Ocean. NASA initiated this collaboration with CNES and the Territoire des Terres Australes et Antarctiques Françaises (TAAF) in 1993. The diaries were maintained by remote duty personnel during the 1993-1994 mission as part of the International Antarctic Psychological Program (IAPP). This project builds upon previous research by the principal investigator concerning the behavioral issues associated with isolation and confinement. The objective of the study is to develop further understanding of the human requirements for long-duration space exploration.

With NASA concurrence, initial work on the project was delayed until April 1997 to permit Dr. Claude Bachelard to return from Antarctica; Dr. Bachelard is medical director of TAAF, the government agency that administers the French scientific stations located in Antarctica and on three remote islands in the South Indian Ocean. Dr. Bachelard was instrumental in TAAF selecting this project from those submitted by NASA in 1993 due to his experience with the analysis of journals maintained during the International Biomedical Expedition to the Antarctic (1980-81).

NOTE: In April 1997, the principal investigator and Dr. Bachelard together translated and reviewed the diaries, and performed the Level I allocation of entries to the list of behavioral issues included in the original research protocol. Further tasks to be conducted during 1997 include, Perform Level II analyses, Quantify and describe the diary content matrix, and Prepare the final report.

The results of this research primarily will be useful to the planners and managers of remote duty stations, on Earth and in space, and to the crew personnel who live and work in isolation and confinement. The potential benefits, however, are not limited to special duty conditions. In a very real sense we are all crew members onboard a space ship, and we might all learn how to better adapt to our conditions, and get along with each other, by studying examples of groups that have succeeded and failed under circumstances far more difficult than our own. In important ways, studying small groups in isolation and confinement is like viewing society through a microscope. There is much of general value to learn from this approach.

Spatial Auditory Displays for Space Missions

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Funding:

Project Identification: 199-06-12-36
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $227,000
Students Funded Under Research: 4

Responsible NASA Center: ARC

Task Description:

An integrated basic research, applied research,m 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 second grant-year includes completion of five psychophysical studies. Five articles or book chapters, three articles in submission, twelve conference papers, one technical report, seventeen technical

presentations, and one CD-ROM also resulted. These experiments investigated localization performance for virtual sources both with and without head and/or source motion, as well as optimal intelligibility of speech sounds as a function of spatial position using the "telephone-grade" audio that is likely to be a characteristic of many real-world systems. In addition, one study was published and a second is underway to determine echo thresholds using non-headtracked stimuli in which the number and salience of early reflections is manipulated. This included the development of software-hardware systems for both prediction and evaluation of the character of the acoustic environment.

Continued work in the area of technology development included refinement of the Crystal River Engineering "Snapshot" HRTF measurement system at NASA Ames, and initial development of an *in situ* surface transfer function measurement system for diffuse field modeling. A modification of the Snapshot system to integrate head-position sensor information into the measurement chain is nearly complete. Progress has also been made on developing software extensions to allow measurement of materials properties. To the extent possible, we also began the development of basic software capabilities to enable the synthesis of reflection cues in dynamic contexts. Initial planning and some software development for this project has been accomplished. The current platform being considered shifts the primary implementation role to software and uses a general purpose CPU as the hardware platform.

The work overall enables the eventual implementation of a fully-functional, real-time implementation of a virtual acoustic environment. Among the major findings so far are: (1) the added computational expense of real-time implementation of head tracking for source and/or head movement is worthwhile if minimization of image 'reversals' is necessary—a reduction in reversal rate from 28% to 7% was found in one experiment; (2) a speech intelligibility advantage of up to 6 dB can still be obtained with narrow-band, telephone grade audio that utilizes a virtual acoustic display; and (3) the threshold level for early reflections in a virtual acoustic rendering system will vary amongst individuals, and is approximately –23 dB below the level of the direct sound for arrival times between three and 15 msec.

The 3-D audio research activities conducted at ARC under this grant 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 Crystal River Snapshot system for measuring HRTFs of individuals in reflective environments. Previous to the development of the Snapshot system, the measurement of HRTFs was very costly in terms of time and equipment, and required the use of specialized facilities such as an anechoic chamber. The Snapshot system enables measurements in normal reflective environments such as an office, and utilizes a standard PC and sound card. Another example is the US patent awarded in 1995 for "Multi-Channel Spatialization System for Audio Signals." This device enables communication personnel to use their inherent ability to segregate, monitor, and switch attention among multiple communication channels (as many as seven radio communication channels are monitored simultaneously during NASA shuttle launch operations). We fabricated virtual acoustic display prototypes based on this patent for both Kennedy and Johnson Space Centers. Desired signal levels can be heard at a lower volume against background noise and intelligibility is improved, contributing towards less fatiguing and safer operations. Recently, several NASA technology transfer centers have been working to license this technology for hardware used in similar high-stress applications, including 911 operator consoles and aviation communications. The technology is also extendible to teleconferencing, a major commercial application area of 3-D sound technology. Yet another example is the room modeling research we have conducted. The goal is to be able to predict the acoustics and noise levels within a structure before it is built using both prediction software for room modeling and auralization hardware. Such a system also enables virtual listening within the modeled room, and comparison with changes in wall materials, number of noise sources, etc. Once a particular

room has been modeled, we can conduct psychoacoustic experiments to determine how to best modify an acoustical situation for a purpose such as noise reduction. Psychoacoustic methods are used to measure speech intelligibility or other parameters, potentially within a modeled space shuttle laboratory or a modeled conference room on Earth. Finally, the basic research we have conducted in head movement and localization allows our auditory displays to include all of the relevant perceptual and acoustic mechanisms that constitute auditory localization, thereby improving human performance within interactive systems. This work provides developers with the means to improve auditory displays for many different applications, especially those within virtual reality. These include teleoperation, telecommunication, human-machine interfaces, simulation, communication, and design and medical facilities.

FY96 Publications, Presentations, and Other Accomplishments:

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Physiological Effects of Decompression-Induced Venous Bubbles

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Solicitation: 93-OLMSA-07
Initial Funding Date: 6/95
Expiration: 6/98
FY 1996 Funding: $95,507
Students Funded Under Research: 2

Task Description:

Venous air bubbles result from moderate (less than 20,000 ft) decompression to altitude. Known consequences are: vascular obstruction, vasoconstriction, diffuse pain especially around joints, inflammation, edema, and recurring injury to the vascular endothelium. Neurological symptoms can result if venous bubbles become arterialized and embolize the central nervous system. Less known consequences involve the release of vasoactive and permeability altering biochemical mediators, especially from the lungs which are the principal target organ for the venous bubbles, and from activated cells, including neutrophils. These mediators include the prostaglandins, thromboxanes and leukotrienes. Astronauts involved with extravehicular activities (EVA) are at risk for decompression 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 quantitation 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 quantitation 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; 5) Completion of the hyperbaric decompression studies for assessment of eicosonoid production in tissue, urine, bronchoalveolar lavage and blood; 6) Near completion of the hypobaric decompression studies for assessment of eicosonoid production; and 7) Adhesion molecule analysis using flow cytometry has shown so far that no change was observed in the polymorphonuclear (PMN) cells CD18 or CD11a ligands, the lymphocyte CD11b and monocyte CD11c, following hyperbaric or hypobaric decompression.

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 include 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 hypobaric 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.

FY96 Publications, Presentations, and Other Accomplishments:

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Carbon Dioxide-Oxygen Interactions in Extension of Tolerance to Acute Hypoxia

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Project Identification: 199-14-17-14
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 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 O_2) in rest and sequential exercise at 50 and 100 W upon arterial hemoglobin O_2 saturation, pulmonary ventilation, and 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. This allowed 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 us 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.

In related work of previous years leading to the present project, experiments of O_2 breathing by humans at 40,000 ft. simulated altitude, and when breathing 8% O_2 in N_2 at one ATA resulted in loss of consciousness. In

each situation addition of CO_2 to the inspired gas restored consciousness by increasing brain blood and O_2 flow. In beginning the present project, varied mental and psychomotor function tests were studied in subject groups in rest and work at different levels of inspired O_2 to establish methods and conditions for detailed study of acute adaptation to acute hypoxia. Baseline studies of hypoxia alone showed significant decreases in mental functions at 0.10 ATA inspired O_2 in rest and in exercise at 50 and 100 W, but not in exposure to 0.12 ATA. Exposure to 4% CO_2 with 0.10 ATA O_2 prevented the hypoxic decrements in mental function. A separate program of breath-by-breath respiratory measurements, and heart-beat-by-beat Transcranial Doppler measurements of Middle Cerebral Artery Blood Flow Velocity were made, along with Pulse Oximetry determination of arterial blood hemoglobin saturation. These and related rapidly repetitive measurement methods have become parts of a series of experiment sub-sets within the Program, with which to derive dynamic relations of rates of change in brain blood and O_2 flow, alveolar gas composition, and arterial O_2 saturation and content.

Study of comparative dynamic and "equilibrium" responses of respiration, arterial oxygenation, end-tidal alveolar carbon dioxide, and brain blood-flow rate was performed with abrupt exposures to inhaled 10% O_2 and 10% O_2/4% CO_2 at rest, succeeded by 50 and 100 Watts leg exercise. Progressive deterioration of arterial oxygenation and brain oxygenation occurred with 10% O_2 alone. Relative to inspiration of 10% O_2, addition of 4% CO_2 elevated respiratory volume, alveolar oxygen arterial oxygenation, and brain blood-flow and oxygenation. The overall result was an improved, stable oxygenation state with normal arterial blood carbon dioxide pressure. Details of dynamic relations, including statistical analyses of all effects, have been derived and subjected to graphic modeling as the baseline for further analysis.

During the present year, the cited full dynamic physiologic monitoring process was used for comparisons of abrupt 12% O_2 and 10% O_2 exposures, with hypoxia alone and with added 4% CO_2. These exposures showed the "Authority of CO_2" in elevating and stabilizing arterial oxygenation and brain oxygen flow, with both 12 and 10% O_2 exposures. Adding 4% CO_2 to 10% O_2 increased arterial hemoglobin saturation 15% above that produced by 12% O_2 alone, without elevating alveolar carbon dioxide.

Progress in the current year has included development of uniform measurement and analytic methods for comparing rates of development and regression of physiologic adaptations to sudden exposures, to different degrees of hypoxia, without and with concurrent hypocapnia. The rapidity and volume and data acquisition for multiple effects, and the requirements of dynamic correlations has exceeded the usefulness of conventional post-experiment data extraction. For the period ahead, a multi-channel digital data acquisition system was acquired.

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 of this research and technology for the common man relate to improved respiratory support procedures in serious disease, and to safety at work in hazardous closed spaces.

FY96 Publications, Presentations, and Other Accomplishments:

Clark, J.M. "Hyperbaric oxygen therapy" in "The Lung: Scientific Foundations." Edited by: Crystal, R.G. and West, J.B. Raven Press/New York, (in press).

Clark, J.M. and Thom, S.R. "The toxicity of oxygen, carbon dioxide and carbon monoxide" in "Diving Medicine." Edited by: Bove, A.A. and Davis, J.C. (in memoriam), 3rd ed. W.B. Saunders/Philadelphia, PA, (in press).

Clark, J.M., Lambertsen, C.J., Gelfand, R., and Hopkin, E.J. (abstract) Analysis of factors that determine rates of recovery from human pulmonary oxygen poisoning in predictive studies V. Undersea Hyperbaric Med., 23(Supp.), pp 10-11 (1996).

Clark, J.M., Skolnick, B.E., Gelfand, R., Farber, R.E., Stierheim, M., Stevens, W.C., Beck, Jr., G., and Lambertsen, C.J. Relationship of ^{133}Xe cerebral blood flow to middle cerebral arterial flow velocity in man at rest. J. Cerebral Blood Flow Metab., 16(6), 1255-1262 (1996).

Gelfand, R. and Beck, Jr., G. (abstract) Transcranial Doppler adaptation to monitor MCA blood flow velocity during immersion and dry conditions while exercising and at rest. Undersea Hyperbaric Med., 23(Supp), p 29 (1996).

Lambertsen, C.J. Final Report: "CO_2-O_2 interactions in extension of tolerance to acute hypoxia" from Environmental Biomedical Research Data Center, Institute for Environmental Medicine, University of Pennsylvania. EBRDC-IFEM, Report 04-20-95, Philadelphia, PA, (1995).

Lambertsen, C.J. and Gelfand, R. (abstract) Comparison of CO_2-induced improvements in arterial SaO_2 during abrupt exposures of human subjects to 0.12 and 0.10 ATA inspired O_2 in N_2 in rest and exercise. Undersea Hyperbaric Med. 23(Supp.), pp 75-76 (1996).

Thom, S.R., Taber, R.L., Mendiguren, I.I., Clark, J.M., Hardy, K.R., and Fisher, A.B. Delayed neuropsychological sequelae following carbon monoxide poisoning and its prophylaxis by treatment with hyperbaric oxygen. Ann. Emer. Med., 25, 474-480 (1995).

Environmental Biomedical Research Data Center

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Funding:

Project Identification: 199-70-27-14
Solicitation: 95-OLMSA-01
Initial Funding Date: 12/95
Expiration: 11/96
FY 1996 Funding: $ 197,382
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 Environmental Biomedical Research Data Center assets and functions have been developed over the past 25 years. Initial data inclusions and analysis consisted of mathematical integration of new and existing information to aid conceptual interpretations and further research planning. The present organized data accumulation, recording, storage, and analyses procedures are computer-based, with hard copy back-up. These continuing paths

of data systems development and analysis have been important during the past year. A major program concerns extraction and time-based analysis of most of the existing human pulmonary oxygen poisoning data at pressures of 1.0, 1.5, 2.0, 2.5, and 3.0 ATA O_2. The multiple measurements include quantitative, analyzable rates of development and recovery of pulmonary mechanical and gas exchange functions. Extraction of individual experiment results encompassing many thousands of measurements has been completed. The ongoing statistical analyses of O_2 poisoning effects in the exposures to prominent degrees of oxygen pressure provide the basis for the planned next phase of construction of oxygen tolerance tables and models, for onset and recovery at different inspired oxygen pressures. Establishment and analyses of a large data base for Doppler-monitored Venous Gas Embolism (VGE) in existing laboratory hyperbaric decompression trials has been completed. New analytic methods have shown mathematically demonstrable correlation of the empirically monitored Doppler VGE index and a theoretical index of gas phase evolution in hyperbaric decompression. The intent during the coming year includes continued refinement in correlations of Data Center decompression stress models and venous gas embolism. This can be joined with validation of new models of hypobaric decompression stress being developed for EVA 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 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.

FY96 Publications, Presentations, and Other Accomplishments:

Clark, J.M., Lambertsen, C.J., Gelfand, R., and Hopkin, E.J. (Abstract) Analysis of factors that determine rates of recovery from human pulmonary oxygen poisoning in predictive studies V. Undersea Hyperbaric Med., 23 (Supp.): pp 10-11 (1996).

Clark, J.M., Skolnick, B.E., Gelfand, R., Farber, R.E., Stierheim, M., Stevens, W.C., Beck, Jr., G., and Lambertsen, C.J. ^{133}Xe cerebral blood flow to middle cerebral arterial flow velocity in man at rest. J. Cerebral Blood Flow Metab., 16(6), 1255-1262 (1996).

Gelfand, R. and Beck, Jr., G. (abstract) Transcranial doppler adaptation to monitor MCA blood flow velocity during immersion and dry conditions while exercising and at rest. Undersea Hyperbaric Med., 23 (Supp.): p 29 (1996).

Lambertsen, C.J. and Gelfand, R. (abstract) Comparison of CO_2-induced improvements in arterial SaO_2 during abrupt exposures of human subjects to 0,12 and 0.10 ATA inspired O_2 in N_2 in rest and exercise. Undersea Hyperbaric Med., 23 (Suppl.): pp 75-76 (1996).

Thom, S.R., Taber, R.L., Mendiguren, I.I., Clark, J.M., Hardy, K.R. and Fisher, A.B. Delayed neuropsychological sequelae following carbon monoxide poisoning and its prophylaxis by treatment with hyperbaric oxygen. Ann. Emer. Med., 25, 474-480 (1995).

Remediation of Biofilms Formed by Bacteria Isolated from Spacecraft Water Systems

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-04-11-43
Solicitation: 95-OLMSA-01
Initial Funding Date:
Expiration:
FY 1996 Funding: $138,601
Students Funded Under Research:

Task Description:

The current year has focused on the development of the imaging system used in the enumeration of attached cells. This system has been verified with multiple experiments to determine the repeatability of the operation. Experiments have been done that show the efficacy of removal of chlorine, ozone, and iodine on attached bacteria. Both chlorine and ozone worked well with regard to the removal of biofilms. Iodine, although able to disinfect the biofilm did not remove attached cellular materials at levels approaching 48 ppm.

Potential applications of this research include tests for bacterial contamination and the water quality of potable, recreational, and industrial water. This research could produce on-line, real-time monitoring tests for field, home, and industrial use that will be easy-to-use, rapid, selective, and sensitive. Furthermore, the measurement and evaluation of biofilm remediation techniques are applicable to industrial water treatment. The proposed technology development will be beneficial to NASA and will lend itself to future commercial applications, thus addressing one of the aspects of NASA's Strategic Plan regarding the transfer of technology to the private sector.

Spaceflight Effects on Microbial Susceptibility to Antibiotics

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Funding:

Project Identification: 199-04-11-20
Initial Funding Date: 2/95
FY 1996 Funding: $158,666

Solicitation: 93-OLMA-07
Expiration: 2/98
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.

During this last year, further bacterial strain stability and optimization testing was completed. A wide variety of bacteria and yeast species were selected and paired against a variety of appropriate antimicrobials with different modes of action. Test microbes were inoculated into selected types of test cards, which were chosen for specific organism-antibiotic combination. These cards were then refrigerated for a period of up to 15 days and processed in the reader incubator at specific time intervals. The purpose of pairing a test organism with a specific susceptibility test card was to obtain the optimal intermediate minimal inhibitory concentration (MIC) value for each antibiotic. Therefore, breakpoint MIC values on either side of the intermediate MIC value, indicating increased or decreased resistance, may then be determined during an inflight experiment.

The development of the manual card reading system for antibiotic susceptibility testing was initiated to perform such testing in the space flight environment. Such technology will allow us to answer a very important question regarding the appropriate antimicrobial therapy in space. Severe constraints on power, weight and volume, maintenance, calibration, and others limit technology available for use in spacecraft. Even though the effort was undertaken to address a specific question for space flight, this technology could be valuable for some Earth applications. For example, this technology could be used in remote settings without access to a comprehensive diagnostic microbiology laboratory. For example, submarines, battlefield, rural areas throughout the world. The automated instrument marketed to perform the antimicrobial testing using the Vitek cards costs about $100 K (it also performs additional functions). In addition, basic findings of the mechanism of action of antimicrobials on human microbial pathogens in space may lead to important breakthroughs to new antimicrobials on Earth. The observed changes in antibiotic susceptibility in space may provide mechanistic insight regarding microbes ability to "combat" antibiotics. The emergence of antibiotic resistance among bacterial pathogens is creating a crisis in public health and has been written about extensively in the lay press. Learning how microbes become more resistant to antibiotics in space may lead to a better understanding of the Earth-bound phenomenon of multiple drug resistant strains of human pathogenic bacteria.

The Effects of Exercise-Enhanced Denitrogenation on Altitude Decompression Sickness (DCS) Protection

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Funding:

Project Identification: 199-04-17-12
Initial Funding Date: 7/95
FY 1996 Funding: $122,000

Solicitation: 93-OLMSA-07
Expiration: 6/98
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 completed at a rate slightly exceeding the planned rate of five per month. Of the 180 planned exposures, 47% (84) have been accomplished as of September 30, 1996; 42% of the way to completion of the three-year program. The number of subject exposures is insufficient to conclude effects; however, the trend shows approximately 40% DCS incidence following a supine resting 4-hour prebreathe. This level of DCS following such a long prebreathe is higher than reported during studies described by NASA in 1984. The current progress appears to be on track for timely completion, although difficulty in acquiring female subjects will slow progress in the near future. 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.

FY96 Publications, Presentations, and Other Accomplishments:

Webb, J.J., Fischer, M.D., Heaps, C.L., and Pilmanis, A.A. Exercise-enhanced preoxygenation increases protection from decompression sickness. Aviat. Space Environ. Med., 67, 618-624 (1996).

Webb, J.T. and Pilmanis, A.A. (abstract) Denitrogenation Time vs. DCS at 30,000 ft. Aviat. Space Environ. Med. 67:667 (1996).

Factors Affecting Decompression Sickness in Astronauts During Extravehicular Activity

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Funding:

Project Identification: 199-14-17-13
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/97
FY 1996 Funding: $98,266
Students Funded Under Research: 1

Task Description:

Decompression sickness (DCS) has not been reported during extravehicular activity (EVA), but ground-based experiments indicate a 20-30% incidence of pain and 2-3% incidence of chokes or cerebral symptoms. While incomplete reporting of DCS during EVA cannot be ruled out, DCS risk may be influenced by environmental and physiological factors some of which are associated with microgravity and which differ from conditions prevailing in ground-based studies. This hypothesis is the basis of our experiments in which we emphasize exercise and terrestrial simulations of microgravity. We measure respiratory nitrogen elimination during 2.5 or 3.5 hrs of preflight oxygen breathing and monitor subjects for precordial Doppler bubbles during 4 hr exposures at 30,000 feet after ascents at 1,000 or 3,500 ft/min. We investigate the effects on respiratory nitrogen elimination and DCS risk of physiological and environmental factors that may affect nitrogen exchange or bubble formation in the body. To date, the overall DCS incidence is about one third in 213 studies. Analysis of nitrogen elimination data by multiple regression and of DCS and Doppler bubble data by logistic regression indicates that exercise during oxygen prebreathe, prebreathe duration, and immersion during prebreathe significantly enhances nitrogen elimination and reduce the incidence of bubbles and DCS. Body weight and terrestrial gravity also appear to be risk factors for DCS suggesting that DCS risk may be inherently lower in microgravity than at 1-G due to adaptations which influence both bubble formation and tissue perfusion. Our studies in the next year will increase the number of trials to confirm current results.

Studies in the 1940s showed that exercising limbs are predisposed to decompression sickness (DCS). Paradoxically, subjects in our EVA simulations develop leg DCS during arm exercise while standing. To test the hypothesis that gravity is a DCS risk factor for the legs, we simulated microgravity in subjects who were recumbent while performing arm exercise. Seated, resting subjects breathed oxygen for 3.5 hrs before ascent at 3,500 fpm to 30,000 ft (shuttle suit pressure) where they performed standard NASA arm exercises to simulate EVA exercise. Control subjects (n=20) stood while experimental subjects (n=21) reclined on a stretcher. Subjects were interviewed at 16 minute intervals for DCS symptoms and were monitored with Doppler for precordial bubbles after flexing each limb. Microgravity simulation did not affect arm DCS but reduced leg DCS from 53% to 6% ($p=0.0024$). Microgravity simulation did not affect bubbles in the arms, but reduced Doppler Grades >2 in the legs from 44% to 6% ($p=0.0091$). These results support the hypothesis that gravity is a DCS risk factor and suggest the following conclusions: (1) DCS susceptibility in Earth-orbit is less than in most ground-based studies; (2) microgravity should be simulated during ground-based EVA studies; and (3) shorter pre-EVA protocols than currently used should be possible in Earth-orbit. These conclusions require confirmation as: (a) most subjects were not their own controls; (b) NASA EVA exercise simulation may be

significantly different in a reclining rather than a standing position; and (c) reclining may be an inappropriate microgravity simulation.

Decompression sickness (DCS) occurs as a result of hyperbaric exposure (diving, compressed air work, hyperbaric medicine) and hypobaric exposure (air or space travel). DCS mechanisms involve the exchange of inert gases and the formation of bubbles in the body. Although the same physiological mechanisms are involved in DCS that results from either hyperbaric or hypobaric exposure, the environmental and physiological conditions can be different and can lead to dissimilar medical outcomes and risks. For example, our experimental results suggest that humans are less susceptible to altitude DCS in space than on Earth because of naturally occurring adaptations to microgravity. Similar observations apply to the effects of immersion in diving (a terrestrial simulation of microgravity) when compared to hyperbaric exposure under dry conditions where gravity effects are present. Our goals are to investigate the roles of physiological and environmental factors that affect inert gas exchange and bubble formation and to develop a comprehensive mathematical description of these effects. An understanding of the fundamental nature of decompression and a mathematical model describing the kinetics of DCS probability will improve the safety and efficiency with which humans can live and work in the useful range of barometric pressures.

FY96 Publications, Presentations, and Other Accomplishments:

Gerth, W.A. and Vann, R.D. A statistical bubble dynamics model of decompression sickness risk for diving and altitude decompressions. Undersea & Hyperbaric Med., 23, A32 (1996).

Gerth, W.A. and Vann, R.D. Development of iso-DCS risk air and nitrox decompression tables using statistical bubble dynamics models. Final Report for NOAA Grant NA46RU0505, (1996).

Gerth, W.A. and Vann, R.D. Importance of VGE formation in statistical bubble dynamics models of altitude decompression sickness risk. Av. Sp. Environ. Med., 67, A7 (1996).

Molecular Damage of Human Cells by X-rays and Neutrons

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Funding:

Project Identification: 199-45-17-17
Initial Funding Date: 5/95
FY 1996 Funding: $230,860
Joint Agency Participation: DoD

Solicitation: 93-OLMSA-07
Expiration: 5/98
Students Funded Under Research: 5

Task Description:

The central goal of this standard ground-based research project is to identify DNA damage induced by low- and high LET radiations at moderate doses. Damage induced by fission neutrons and by 1 GeV/n Fe ions will be compared to that induced by isoeffective effective x-ray doses in human immortalized cells originating from different tissues. Recent data suggest that exposure to ionizing radiation results in genetic instability, which is expressed as an increase in the levels of transforming, mutagenic, and cytogenetic damage many cell generations after radiation exposure. Our working hypothesis is that radiation response principally occurs in the early stages of carcinogenesis or other radiation-induced pathologies. In the cellular models proposed here, this early phase has a duration of about 10-12 cell divisions or about 15 days. We will study radiation-induced genetic alterations at several time points within the first 2 weeks post-irradiation using several established or novel techniques presently used in our laboratories for studying molecular changes associated with human malignant disease. 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. In addition, we will assess persistent 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 and HZE Fe ions are important components of the space radiation environment. A sizeable proportion of the dose behind spacecraft or lunar shielding is due to neutrons. Neutron irradiations will be performed at the TRIGA Reactor Facility of the Armed Forces Radiobiology Research Institute (AFRRI) in Bethesda, Maryland while Fe ion irradiations will be provided at the AGS facility at Brookhaven National Laboratory.

The overall objective of our program is to characterize in molecular terms the genetic alterations induced by ionizing radiation. Our FY96 specific aims in pursuit of this goal included: (1) performing our first experiments at BNL—obtaining a radiobiological characterization of the Fe ion beam using our test systems; (2) refining and applying our novel differential gene expression assay to acquire a database detailing determination, identification, and characterization of genes differentially expressed by high- and low-LET radiation; and (3) continuing studies of site specific genomic instabilities with special attention to mismatch repair mutations induced by ionizing radiation.

We hypothesize that residual damage remaining in cells offers the best opportunity to observe a molecular radiation signature. We further hypothesize that a molecular signature from high LET radiation will be different from a molecular signature from low-LET radiation. To investigate these hypotheses, we have used x-rays, fission neutrons, and 1 GeV Fe ions to investigate cellular responses at various times up to 2 weeks post-irradiation. Molecular endpoints have included protein and mRNA expression levels, as well as the induction of microsatellite mutations.

The aim of our gene expression studies is to identify genes involved in radiation response at the cellular level, including neoplastic transformation. By screening approximately 5000 cDNA clones, we isolated more than 25 independent clones differentially expressed following irradiation of both HL60 and MCF7 WT cells.

HL60 cells have been characterized as radiation-sensitive (Do < 1 Gy). Exposed to 20 Gy of X rays, they were viable for up to 12 hours. Of 5 candidate radiation-responsive genes, the expression of one gene, Csa-19, was characterized in detail. The abundance of Csa-19 mRNA deceased dramatically in HL60 cells three hours after X-irradiation. The same transcription pattern was observed in MCF7 WT cells, and after exposure of HL60 or MCF7 WT cells to 1.2 Gy of fission neutron-irradiation. Our result that Csa-19 is similarly repressed in p53 normal and abnormal cells provides a new example of an immediate-early gene that is transcriptionally independent of p53, in contrast to other previously discovered radiation-responsive genes (such as for example, gadd45, WAF-1/Cip1, mdm2).

We have now identified 20 other new radiation-responsive genes at a time point 1 week after X- or fission neutron irradiation of MCF7 WT and HL60 cells using our novel unbiased differential cDNA library screening assay. The change in transcription of some of these genes in Fe-irradiated MCF7 WT cells has been compared with the change in transcription of corresponding genes in X-irradiated or fission neutron-irradiated cells (at 20 or 1.2 Gy, respectively). So far there have been no positive results for Fe ions at 2 Gy, probably because the dose was too small; the RBE for survival of these cells was concurrently measured to be 1.3. Additional examples of interesting genes differentially expressed following X-irradiation are the triosephosphate isomerase (TPI) gene required for cell growth and maintenance, and the L-12 gene, associated with EF-1 and necessary for fidelity of translation and kinetic proofreading. Two of the known genes isolated in fission neutron-irradiated cells, L-23, and TI-227, one week post irradiation have been reported by other groups in association with tumorigenicity and metastatic potential. Accordingly, the L-23 gene is implicated in genomic imprinting process and, thus, could be involved in multigene human diseases such as breast cancer. The postulated cellular function of Tl-227 is to regulate the expression of various genes as a transcription factor in the complex process of metastasis. All identified genes were nuclear, except oxidase II (mitochondrial). For remaining genes, no match has been found in the public databases. Attempts to characterize both novel and known genes discovered in this laboratory are currently underway.

We are also investigating the possibility that radiation can readily induce deletions in short oligonucleotide repeat sequences known as microsatellites. Such mutations could affect mismatch repair and are associated with many diseases including cancer. Assuming that moderate to high doses of radiation result in detectable yields of such mutations, we subjected numerous aliquots of DNA from irradiated cells to polymerase chain reaction (PCR) amplification using microsatellite primer sets. The amount of DNA in each aliquot was equivalent to that contained in only several cells, so that a mutation in only one of the several cells might be detected. Preliminary studies with human pre-leukemic HL60 cells irradiated with 20 Gy of X-rays have identified mutations at the BAT26 locus on chromosome 2. These results are being confirmed by study microsatellite induction in cell lines known to be proficient and deficient in mismatch repair. These studies will be extended to other microsatellite loci and to fission neutrons and Fe ions.

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, and were at 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 atherosclerosis, increase of leukemia and thyroid gland cancers, and premature aging of the immune system as well as the whole organism. A specific point we attempt to resolve by performing these studies concerns the ability to factor out a radiogenic contribution to health effects from contributions resulting from complex interactions between radiation and other harmful agents 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 to the two cell types. Our studies are designed to define cellular transformation in molecular terms by characterizing the altered genetic program induced by exposure to radiation. Knowledge of radiation-specific gene damage will be most valuable for the purpose of radiation protection as well as litigations involving radiation as a causal agent of a disease. Finally, antagonists or agonists of radiation-specific gene therapy could be applied in future advanced molecular therapies or preventive strategies, such as being currently developed for other specific human populations at risk (e.g., those at risk 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 (e.g., specific examples here are astronauts exposed to both low- and high-LET environment, 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 radionucleotides including α-, ß- and γ- emitters. This situation persists for protracted durations due to the 30-year half-life of 137Cs and 90Sr and the more than 10,000 year half-life 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. We are introducing a novel strategy for screening a very large number of possible gene targets regardless of the normal level of transcription in the cell. This method consists of differential screening of HL60 cDNA library. The primary cDNA library has 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 lies 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 polymerae chain reaction (PCR)-amplified cDNA inserts (thus, a gel containing 20 lanes with each lanes containing 10 amplified inserts possess 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).

FY96 Publications, Presentations, and Other Accomplishments:

Balcer-Kubiczek, E.K., Zhang, Q.-Q., Zhang, Z.-F., Shi, Z.-M., Harrison, G.H., Ordonez, J.V., Meltzer, S.J., Hickey, R.J., and Malka, L.H. Alteration of proteins comprising the replication complex in X-ray survivors. Radiation Research Society Annual Meeting, abstract 920-356 (1996).

Harrison, G.H. Local vistas in radiation molecular biology. Grand Rounds in the Department of Radiation Oncology, University of Maryland, Baltimore (September 4, 1996).

Harrison, G.H. and Balcer-Kubiczek, E.K. Consequences of non-uniform irradiation of defined target volume. Forty-fourth Radiation Research Society Annual Meeting, abstract 909-156 (1996).

HZE and Proton-Induced Microenvironment Remodeling

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Funding:

Project Identification: 199-45-17-25
Solicitation: 95-OLMSA-01
Initial Funding Date: 7/96
Expiration: 9/96
FY 1996 Funding: $ 192,426
Students Funded Under Research:

Task Description:

The cosmic radiation environment is a complex mixed radiation field. This proposal will contribute an experimental design that focuses on quantitative assessment of specific tissue effects induced by important components of the space radiation environment: protons and iron ions. Determining the risk from radiation exposure during space travel is constrained by the lack of understanding of basic mechanisms of radiation effects on multicellular tissue processes that lead to functional impairment and carcinogenesis. We have identified microenvironment remodeling as a novel early endpoint of radiation exposure and have recently found certain effects that appear to be dependent on radiation quality. The microenvironment of tissues encompasses insoluble extracellular matrix proteins and soluble growth factors. Our studies in mammary gland have demonstrated that radiation-induced microenvironment alterations are rapid (<24 hr), persistent (>7 days), and sensitive to low doses (<50 cGy). The proposed studies will evaluate the generality of this novel tissue process and of its HZE dependence. Identification of tissue-specific remodeling will provide fundamental knowledge of radiation effects on selected tissues, generate correlations between events in tissue remodeling, determine which events are tissue-specific or radiation quality dependent, and quantitate these events for correlation with radiation fluence or dose. The specific goals of this project are to evaluate early (~15 min - 14 day) temporal and spatial changes in the composition of the irradiated microenvironment as a function of tissue type, radiation quality and dose or particle fluence. Liver, skin, Harderian gland and brain from animals whole body irradiated with 600 MeV and 1 GeV Fe particles, 200 MeV plateau protons or 60Co gamma-radiation will be evaluated. Selected changes will be quantified using image analysis and dose response relationships will be determined, with an emphasis on doses of less than 1 Gy. Understanding the mechanisms of tissue response will contribute to risk assessment and may lead to new strategies for intervention.

The objective of the proposal is to evaluate early events leading to changes in the composition of the irradiated microenvironment as a function of radiation quality and dose at various times postirradiation and to compare these changes between different tissues. Our first goal was to develop an image analysis program to quantify the previously observed disruptions of the basement membrane of the mammary gland. An interactive program called *Xrad* was developed by co-investigator, Bahram Parvin, that allows the experimenter to identify morphological objects of interest for computer automated segmentation of the basement membrane. The program then uses a deformable contours approach to greatly refine the initial rough outline of the membrane. The final result is outlined membrane that has been segmented much more rapidly and precisely than can be done

manually. The initial approach to define specific changes in the character of contour was based on the determination of smoothness. *Xrad* determined the average curvature, standard deviation of average curvature, average of the curvature maxima, and length of contour divided by number of curvature maxima. However, the analysis of membrane smoothness did not reveal a parameter that tracked with dose. We are now attempting to define other segment characteristics based on further image analysis. Our second objective was to evaluate the tissue dependence of radiation induced changes in the microenvironment. Current data obtained from analysis of gamma-irradiated liver show tissue-specific response, which indicates that cell type and multicellular interactions play a prominent role in determining the character and compositon of radiation-induced microenvironment remodeling. In addition, during BNL-2 we collected mammary gland, liver, and skin from animals irradiated with doses ranging from 0.03 to 1.6 Gy at one hour and 12 hour post-irradiation.

Our basic research is directed towards an understanding of factors that influence the progression of neoplastic disease. Factors that influence neoplastic transformation, the escape from normal regulatory controls, and the rate of progression of the initiated cell *in vivo* are poorly understood. New knowledge of the critical role that microenvironment plays in eliciting appropriate function in normal cells is the basis for targeting radiation-induced microenvironment alterations for understanding how the microenvironment in which the initiated cell finds itself can dramatically influence its ability to express the altered phenotype. By studying different radiation qualities, which as evidenced by our preliminary studies elicit different types of microenvironments, we can further refine our understanding of how microenvironments affect the develpment of cancer. A potential benefit of this research is the possibility to design therapeutic interventions to interrupt this process before frank malignancy is evident.

Lens Epithelium and Proton-Induced Cataractogenesis

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Funding:

Project Identification: 199-45-17-14
Initial Funding Date: 2/95
FY 1996 Funding: $198,736

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 5

Task Description:

Cataracts are a potential late side-effect of space travel that impacts risk assessment and spacecraft design. Presently, there are inadequate data to estimate accurately the risk of radiation-induced cataract in man at the relatively low particle exposures anticipated for space flight. Cataracts also arise in uveal melanoma patients as a complication following their successful treatment with proton or helium radiotherapy. We have been studying the relationship between the calculated helium-ion exposure of specific sublenticular volumes and the later appearance and location of cataracts. The objective of the proposed research is to determine the proton-induced alterations in chromosomes and in protein expression that are important to cataractogenesis, and ultimately to develop strategies to diminish the incidence or severity of these changes. We will test a hypothesis that radiation-induced changes in protein expression are important in cataract formation. To this end, experiments have been designed with three specific aims: 1) Characterize the acute radiation response of cultured human cells of the lens epithelium grown on extracellular matrix. Quantitative dose-response measurements between proton- and x-ray-induced survival, and yields of micronuclei and chromatin breaks and rejoining will be determined. The fidelity of chromatin repair will also be assessed; 2) Radiation is known to induce basic Fibroblast Growth Factor (bFGF) in cultured endothelial cells and this cytokine is associated with changes in the radiation response. Experiments are proposed that will determine whether protons or x-rays change levels of bFGF mRNA or protein in the lens epithelial cells; and 3) An investigation of the possible modification of intracellular lens proteins by protons is proposed. The proposed work will elucidate relationships between proton-induced damage to the chromatin of lens epithelium *in vitro*, and biological consequences to the cells surviving the resulting damage. This knowledge will allow correlative comparisons with available experimental work *in vivo*, and may provide a basis for elucidating the biological mechanisms contributing to cataractogenesis and to improved approaches to estimate risk of cataract due to exposures in space travel.

We have completed six major proton studies during the last year of funding with the primary human lens epithelial (HLE) cultures to continue our proposed series of studies on their radiation response. We have made progress in developing a cell survival assay for the HLE cells by using the bovine corneal endothelial (CE) cells as a feeder layer. The CE cells are used by us to make the extracellular matrix (ECM) which is essential for the growth of the primary HLE cells *in vitro*. The confluent CE feeder cells were irradiated with a high dose of x-rays which leaves them unable to divide but able to provide supporting nutrients to the HLE test cells. Within 24 hours, the HLE cell population, which received graded doses of protons, were trypsinized and seeded onto the irradiated CE feeders. After two weeks to allow for clonal growth, the cultures were pulse-labeled with

3H-thymidine to permit incorporation of the isotope into the DNA of viable cells in the resulting clones. Quantitative autoradiography is in progress to score the percent surviving the proton doses. Two experiments measuring proton- and x-ray-induced micronuclei and the yield of chromatin rearrangements in the HLE are being processed for correlation with the survival studies.

We have measured the human HLE cytokine bFGF using the QuantikineTM immunoassay kit from R&D Systems in pre- and post-irradiated media from bFGF fed and bFGF minus HLE cultures. Since this system measures unbound bFGF it is useful as one tool to assess the availability of bFGF in the media. HLE cultures passage #P3-#P7 are used in the studies. Our initial studies of cell growth and differentiation with days-in-culture have been conducted with the cells maintained at 37° C and 10% CO_2 and fed every other day with media containing 5 ng/ml bFGF. ELISA studies of unbound bFGF confirm that feeding every other day are needed to keep bFGF levels from depletion. Preliminary measurements show that cultures not fed bFGF for 24 hours prior to irradiation had zero unbound bFGF in the media either pre- or post-irradiation up to 24 hours after irradiation. However, with feeding cultures as prescribed, measurements of bFGF were never lower than 200 pg/ml, and in a time course study, were somewhat elevated at 6 hours after irradiation with a 4 Gy dose. This time course also coincides with the bFGF mRNA signal that is induced after a 4 Gy dose of protons in confluent, fed populations. This result may indicate that some bFGF is needed in order for the cells to make more to protect them from the irradiation. Survival studies are underway to determine what levels of bFGF are needed minimally to give this protection. We have been working to enhance our resolution of this proton-induced effect by switching from our oligo bFGF probe for our Northern studies to a cDNA probe. This transition is still in progress but should allow a more sensitive completion of our time course effects.

Our previous studies had indicated that HLE cells after 3 days in culture (after inoculated at 5 X 105/35 mm dish) show evidence from SDS-PAGE combined with Western blots for the expression of a- and b-crystallin, but no g-crystallin which did appear in cultures that were 2 months old. The g-crystallin is considered a marker for differentiation of the HLE cells into lens fiber cells. During the past funding period we have significantly filled in our time course studies. Our new results now indicate that at 3 wks in culture, there is a huge g-crystallin signal on the immunoblots from the HLE cultures, that actually wanes to the g- crystallin levels we detected after 1.5, 2.0, 2.5 and 3.0 months in culture. At three weeks in culture, a-, and b-crystallin signals are still detected but are significantly less than the g-crystallin levels. A human fibroblast (not derived from lenticular tissue) was used as a control cell and purified a-, b-, and g-crystallin standards (of bovine origin) were run as molecular weight markers. The antisera to a-, b-, and g-crystallin were kindly provided by Dr. Sam Zigler (NEI) and were raised in rabbits against bovine crystallin and have good cross-reactiviity to the human crystallin proteins. We find the 3-week in culture time point to be a pivotal culture age for not only the achievement of confluence in cell density and the appearance of g-crystallin and other morphological markers of differentiation, but also the expression of proton-induced expression of bFGF. We are focussing our continued studies on 3-week old cultures and looking at changes in the lens crystallin profiles after irradiation by protons and xrays.

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 the particle radiation-induced cataract indicates the extent of our lack of data and has only heightened the level of uncertainty in assessing radiation risk. Some intriguing new data on the inhibition of radiation cataractogenesis by the aminoalkyl phosphorothioate analog WR-77913 provides incentive to the pursuit of cataract countermeasures, and may reveal the role of other critical targets of damage in the eye (e.g., the ciliary body) that impact the expression of lens damage.

Irradiation of the young mammalian lens causes mitotic arrest followed by apparent excess mitosis with production of fragmented nuclei and degenerate cells. Cataracts can be induced by lower doses of high linear energy transfer (LET) radiations compared to x- or g-rays. Disorganization in the meridional row and the frequency of abnormal mitoses and micronuclei are related to both the fluence (number of heavy particles/unit area) and also to the LET of the charged particles. At a given dose, as the LET of the radiation increases, the number of abnormal mitotic figures, micronuclear frequency, and disorganization of the meridional row also increases. The severity of the meridional disorganization and micronuclei number go up with the increasing fluence or dose for particle of the same LET. Fractionation of the charged particle irradiation does not produce dose sparing, and in some cases produces a dose-dependent enhancement in the incidence of cataract. These data support a generally accepted hypothesis that radiation cataractogenesis is the result of genomic injury to the lens epithelial cells. Analysis of the occurrence of posterior lenticular cataracts in patients treated with low-LET radiation for cataracts had in the past led to the commonly accepted threshold dose of 2 Gy for cataract induced by a single acute exposure. A new technical report has been published that reexamines the incidence of cataracts seen in the years 1949-64 among 2249 Hiroshima atomic-bomb survivors with known Dosimetry System 1986 (DS 86) doses. Among several dose-response relationships with or without two thresholds, the best fit based on binomial odds-regression models is achieved with a linear-linear dose-response relationship that assumes different thresholds for neutrons and gamma-rays. The estimates of the two 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 countermeasures to avoid the risk where possible in medical treatments or occupational exposures.

Potential benefits to be gained by the development of the proposed research plan include a more realistic estimate of the risk of radiation-induced cataract that could impact the design of payload requirements or operational limitations including extra-vehicular activity for flight missions.

Proton Radiation Studies

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-45-17-04
Initial Funding Date: 7/95
FY 1996 Funding: $200,000
Joint Agency Participation: DoD (USAF)

Solicitation:
Expiration: 6/97
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-three 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; and 3) analysis of cataract data from the subjects plus other species (including humans) to extrapolate deterministic effects more effectively to humans. Projects depending on continued NASA support of the subjects include 1) evaluation of genetic damage by measuring persistent chromosome translocations, and 2) continuing measurements of radiogenic cataracts. Expected results include information on late stochastic and deterministic effects, and chromosome aberration dose response curves, both of which will be relevant and applicable to space radiation risk estimates and to "biodosimetric" analysis of cells from humans exposed to unknown radiation doses.

The long-term project, "Proton Radiation Studies" is now in its 33rd year, and 14 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 (9/14) 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 live 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 future study (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 1945, 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, 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 started 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 data will be applicable to human risk estimates for aging as well as for space radiations.

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 was held on March 6, 1996. Topics covered included 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.

FY96 Publications, Presentations, and Other Accomplishments:

Cox, A.B. and Hardy, K.A. (abstract) The space radiation environment: Hazards for humans and hardware outside the earth's magnetosphere. 26th International Conference on Environmental Systems, Monterey, CA, 8-11 July 1996.

Cox, A.B., Fanton, J.W., Troter, R.W., DiCarlo, C.D., Mason, P.A., Hardy, K.A., Hanes, M.A., Leavitt, D.D., Lucas, J.N., Lett, J.T., Williams, G.R., and Lee, A.C. (abstract) Nonhuman primates exposed to energetic protons: A 32-year study. Third BELLE Conference, NIEHS, Research Triangle Park, NC 12-14 November 1996.

Cox, A.B., Hanes, M.A., and Lett, J.T. (abstract) Effects of low and intermediate doses of particulate radiations on long-lived animals: Late stochastic and deterministic effects. 31st COSPAR Scientific Assembly, Birmingham, UK, 14-21 July 1996.

Cox, A.B., Hardy, K.A., Lett, J.T., Lee, A.C., Williams, G.R., Hanes, M.A., Fanton, J.W., Leavitt, D.D., Mason, P.A., and Lucas, J.N. (abstract) Late-effects studies in irradiated primates: Radiobiology and dosimetry related to risk estimates for space flight and Space Station. 26th International Conference on Environmental Systems, Monterey, CA, 8-11 July 1996.

Hanes, M.A. and Cox, A.B. (abstract) Pathology of late proton effects in nonhuman primates. 31st COSPAR Scientific Assembly, Birmingham, UK, 14-21 July 1996.

Lucas, J.N., Hill, F.S., Burk, C.E., Cox, A.B., and Straume, T. Stability of the translocation frequency following whole-body irradiation measured in rhesus monkeys. Int. J. Radiat. Biol., 70 (3), 309-317 (1996).

Human Enzymatic Repair of Radiation-Induced DNA Breaks

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Project Identification: NAGW-4396
Initial Funding Date: 2/95
FY 1996 Funding: $147,408

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 3

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 previously determined the mutagenic potential of DNA strand breaks in the shuttle vector pZ189 in human fibroblasts. The bleomycin conditions used produce strand breaks with 3'-PG termini as >95% of the detectable dose-dependent lesions. Breaks with this end group represent 50% of the strand break damage produced by ionizing radiation. We found that such strand breaks are mutagenic lesions.

The type of mutation produced was largely determined by the type of strand break on the plasmid (i.e. single 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 of DNA damage, biological outcome should be 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.

The mutagenic potential of DNA strand breaks in cells defective in their strand-break repair capacities. Assessment of biologically relevant endpoints in cells defective in specific proteins is a powerful tool to assess the role of those proteins in determining the biological endpoint. In our case, we sought to determine whether cells deficient in proteins that are known to be responsive to DNA strand breaks had altered mutagenic potential for strand-break mutagenesis.

The fidelity of double-strand-break repair was compared in ataxia-telangiectasia (A-T) fibroblasts. The A-T cells are radiosensitive and contain a defect in a single gene (ATM). The predicted gene product of ATM has homology with DNA-dependent protein kinase (DNA-PK). The A-T cells showed a two to three-fold increase in mutagenesis compared to the normal fibroblast cell line, WI-38. These results suggest that loss of repair fidelity may contribute to some of the phenotypes observed in these cell lines, such as their cellular radiosensitivity, and perhaps the cancer proneness seen in A-T. Cellular A-T phenotypes, such as radiosensitivity and genomic instability, suggest that a deficiency in the repair of DNA double-strand breaks (DSBs) may be the primary defect; however, overall levels of DSB rejoining appear normal. We used the shuttle vector, pZ189, containing an oxidatively-induced DSB, to compare the fidelity of DSB rejoining in A-T and normal fibroblasts. Mutation frequencies were not only higher, but also the mutational spectrum was different. The deletions in plasmids recovered from normal cells were always between short direct repeat sequences, implicating illegitimate recombination in DSB rejoining. Deletions in A-T did not occur at direct repeats, suggesting a defect in illegitimate recombination. These findings suggest that the A-T gene product may either directly participate in illegitimate recombination or modulate the pathway. Regardless, this defect is likely to be important to a mechanistic understanding of DNA double-strand break repair.

The majority of known human carcinogens have been shown to be potent mutagens, and mutagenesis is thought to be the principle mechanism by which cancer is initiated. Ionizing radiation is a known mutagen, and mutation of key target genes within irradiated cells is probably the initial (irreversible) event which starts a cell on the pathway to tumorigenesis. Cellular DNA repair systems can both mitigate and potentiate the mutagenic consequences of radiation-induced DNA damage through a variety of "error-free" and "error-prone" repair pathways. Understanding these pathways, and the environmental factors that influence them, is probably key to understanding the mechanisms of mutagenesis and cancer induction in man, as well as the cancer risk associated with radiation exposure.

FY96 Publications, Presentations, and Other Accomplishments:

Dar, M.E., Winters, T.A., and Jorgensen, J.J. (abstract) Identification of defective illegitimate recombinational repair of oxidatively-induced DNA double-strand breaks in ataxia-telangiectasia cells. 45th Annual Meeting of the Rad. Res. Soc.

Mutations in Human Lymphoid Cells

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Funding:

Project Identification: 199-45-17-06
Initial Funding Date: 5/95
FY 1996 Funding: $304,518

Solicitation:
Expiration: 4/97
Students Funded Under Research: 4

Task Description:

The goals of this proposal are to determine the susceptibility of human cells to mutagenesis following exposures to charged particle radiations found in space. We are studying the heritable alterations produced in human lymphoblasts following exposures to protons or iron ions. Protons are the dominant component of the space radiation environment. While iron ions are much less abundant, they are thought to be much more damaging to cells and tissues due to their energy deposition characteristics. We will determine the effects of certain biological variables (e.g. gene copy number, genetic linkage, and the expression of genes that may regulate the stability of the human genome) on cellular susceptibility to mutagenesis and cytotoxicity following exposure to low doses of protons or iron ions.

These studies are being performed with syngeneic human B-lymphoblastoid cell lines that are respectively expressing either only normal p53 or only mutated p53. The p53 tumor suppressor gene is mutated in a wide variety of human tumors. In addition, mutations in p53 are often associated with an increased resistance to the toxic effects of ionizing radiations. We have shown that cells expressing mutant p53 are indeed more resistant to the toxic effects of either protons or densely ionizing iron ions. In addition, cells expressing mutant p53 are more easily mutated by either protons or iron ions than cells that express normal p53 protein. The increase in mutation susceptibility is much more pronounced for the autosomal tk locus than for the X-linked hprt locus.

Molecular analysis of mutations will provide information on the types of heritable DNA structural alterations that result from the traversal of human cells by low fluence exposures to Fe ions and to moderate doses of protons. The characterization of mutant spectra is ongoing in both the p53 mutant cell line and in the cells that express normal p53.

The major highlight of our studies in FY96 was the initiation of experiments with 1087 MeV/amu Fe ions at the Alternating Gradient Synchrotron. We successfully carried out mutagenesis experiments using this beam in syngeneic human lymphoblasts that express either normal or mutated p53 protein. The TK6 cell line expresses normal p53 protein while the WTK1 cell line expresses homozygous mutant p53. Physical characterization of the radiation geometry used demonstrated that the LET in the sample position was 146 keV/μm.

In these first studies we showed that Fe ions produce different levels of mutations in the paired cell lines. In the case of the autosomal tk locus, the p53 mutant cells were 25-45 times more sensitive to Fe-induced mutation than were the syngeneic cells expressing normal p53 protein. For the X-linked hprt locus, the p53 mutant cells were more susceptible to mutation, but the magnitude of the response was very different. Only a two-fold enhancement in mutation susceptibility was observed as compared with the cells expressing normal p53.

These initial studies will allow us to carry out further experiments in the Fall of 1996 where we expect to collect independent Fe-induced mutants for molecular analysis of mutant spectra. We plan to collect tk-deficient mutants and hprt-deficient mutants that arise in cells expressing normal p53 as well as from cells expressing mutant p53.

Another highlight of our progress in FY96 was the completion of a large linkage mapping study on 304 hprt-deficient mutants collected after exposure to low fluences of particles ranging in LET from 32-190 keV/μm. Individual mutant DNAs were analyzed for loss or retention of 14 probes that have been mapped on the long arm of the X chromosome. The major findings can be summarized as follows:

1) Based on our cell killing data, we expect that the majority of the hprt mutations analyzed arose as a consequence of a single particle traversal of the X chromosome.

2) Deletions were evident in 70% of the mutants analyzed.

3) Deletions were as small as 15 basepairs and as large as 3 million basepairs.

4) Only one mutant out of more than 300 analyzed showed loss extending beyond the marker 342R, located 1.26 million basepairs telomeric to hprt. No mutants lost the next flanking marker, 529R, located 1.4 million basepairs telomeric to hprt. These data strongly suggest that there is a gene required for viability of the lymphoblastoid cells that is located between these two markers.

5) Discontinuous deletions were seen in 8/213 deletion mutants. These discontinuous deletions are a hallmark of mutations arising after exposure to densely ionizing radiations as none were observed in a similar series of X-ray-induced hprt mutants nor were any observed in another series of spontaneously arising hprt mutants.

6) Genomic sequencing of a limited number of hprt-deficient mutants arising after exposure to iron ions or silicon ions suggests the involvement of short direct or inverted repeats in the formation of deletion junctions.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Heilbronn, L., Kronenberg, A., Ludewigt, B., Miller, J., Nyman, M.A., Singh, R.P., Zeitlin, C., Lazarus, D., McGahern, W., Sutherland, B.M., and Vazques, M. A new facility for radiobiology with high energy heavy ions. 44th Annual Meeting of the Radiation Research Society, Chicago, IL (1996).

Kraemer, S., Kronenberg, A., Ueno, A., and Waldren, C.A. Measuring the spectrum of mutation induced by HZE and low LET radiations in the human-hamster hybrid cell line ALC. 44th Annual Meeting of the Radiation Research Society, Chicago, IL (1996).

Kronenberg, A., Gauny, S., and Cherbonnel-Lasserre, C. Effect of p53 status on cell killing and mutation induction following low fluence exposure to 1090 MeV/amu Fe ions. Seventh Annual Investigator's Workshop in Space Research, Riverside, CA (1996).

Kronenberg, A., Gauny, S., and Cherbonnel-Lasserre, C. Fe ion mutagensis of syngeneic human lymphoid cell lines differing in p53 status. 31st Scientific Assembly of the Committee on Space Research (COSPAR), Birmingham, UK (1996).

Kronenberg, A., Gauny, S., and Cherbonnel-Lasserre, C. Susceptibility of syngeneic human lymphoid cell lines differing in p53 status to cell killing and mutation induction following low fluence exposure to 1090 MeV/amu Fe ions. 44th Annual Meeting of the Radiation Research Society, Chicago, IL (1996).

Kronenberg, A., Gauny, S., Cherbonnelc-Lasserre, C., Vannais, D., Ueno, A., Kraemer, S., and Waldren, C.A. Mechanisms of ionizing radiation-induced mutagenesis. In Radiation Research 1895-1995 (eds. Hagen, U., Harder, D., Jung, C., and Streffer, C.), Volume 2, Congress letters, Proceedings of the Tenth International Congress of Radiation Research, Wurzburg, Germany, pp. 535-538 (1995).

Nelson, S., Grosovsky, A., Gauny, S., and Kronenberg, A. Molecular characterization of 304 hprt-deficient mutants of human lymphoid cells arising after low fluence exposures to high energy heavy ions. Seventh Annual Investigator's Workshop in Space Radiation Research, Riverside, CA (1996).

Waldren, C., Vannais, D., Drabek, R., Gustafson, D., Kronenberg, A., and Ueno, A. Mutant quantity and quality in human-hamster cells (AL and AL -179) exposed to 137Cs-g, protons and HZE-Fe. Adv. in Space Res., (in press).

Radiobiological Studies - Task V (Final Year)

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Project Identification: 199-45-17-07
Initial Funding Date: 7/95
FY 1996 Funding: $50,000

Solicitation:
Expiration: 3/97
Students Funded Under Research: 1

Task Description:

The project was completed as planned, except that one retina from each of the very old animals (controls and irradiated subjects) has been archived in liquid nitrogen in the anticipation that a complementary method of molecular biological analysis for radiation-induced genomic instability in the terminally differentiated, post-mitotic cells of the central nervous system (CNS) will become available in the not-too-distant future.

Surviving animals were scored for cataracts until they became moribund, or were otherwise recommended for euthanasia by attending veterinarians in the Painter Animal Facility at Colorado State University. One final, cross-calibration of lenticular opacification was conducted. Analyses of DNA damage in photoreceptor cells and their losses from the retinas of sacrificed animals were conducted as described previously, except as noted above. Overall analyses of the changes in the size distributions, etc., of the photoreceptor DNA will be undertaken in due course to the extent that funding permits. Reports on some of the findings were presented at the 31st COSPAR Scientific Assembly in Birmingham, U.K. in 1996.

Under a separate program, preliminary efforts have begun at extrapolation across species (rats, rabbits, dogs, monkeys, and to humans) for radiation cataractogenesis using data collected over the past 25 years. Those efforts are, and will be, concomitant with and complementary to a project at the Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki.

Note that the experiments on retinal photoreceptor cell populations, which have been conducted over a period of approximately two decades with ionizing radiations of different qualities, have examined *in situ* a process that recently has been called genomic instability. As far as can be ascertained, this is the first recorded study of that phenomenon in "neuronal" cells of the CNS.

For about 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 into concurrent investigations in other laboratories, both nationally and internationally, with different animal models, especially non-human primates, and other radiations, especially protons. Even more important is the recent extension to humans of the studies of radiation cataractogenesis through a project at RERF, which is funded under a separate NASA program and is conducted in collaboration with Japanese and U.S. scientists at that facility. In this way, it should 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, has been completed, except as noted previously. It seems likely that information of this nature can also be extracted from the data being collected currently at RERF. From the experiments conducted to date, the following conclusions can 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 lifetimes, long after their mission careers are over. At such times, the late, progressive radiation cataracts 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 CNS could cause not only loss of "neuronal" function (circuitry and/or logic) in astronauts late in life but also could affect their performances during the missions.

3. Damage to the CNS caused by exposure to galactic heavy ions that could seriously affect performance during an extended mission beyond the protection of the geomagnetic field (and possible loss of the mission) is likely to be the only biological radiation hazard of concern for NASA before the year 2050 or even the year 2100, unless the geopolitical situation deteriorates markedly in the interim. Such late stochastic sequelae as cancers and the biological changes preceding carcinogenesis, i.e. genomic instability and mutagenesis, will be effectively irrelevant during those time periods. Note that damage to the DNA in the terminally differentiated, nondividing cells of the CNS is a nonstochastic (deterministic) effect.

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) resulting from participation in extended missions. The expectation that this objective will be achieved has been enhanced recently by the initial phase of a separate study in which human radiation cataractogenesis is evaluated from medical information.

2. An evaluation of damage induced 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 (CNS).

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 CNS, the possible effects of terrestrial environmental radiation on the CNS, and the consequences of damage to the CNS 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 "neuronal"cell populations in the CNS. Studies of radiation-induced genomic instability in much simpler systems now seem to be coming into vogue in research programs funded by NASA and other federal agencies.

FY96 Publications, Presentations, and Other Accomplishments:

Cox, A.B., Hanes, M.A., and Lett, J.T. Effects of low and intermediate dose of particulate radiations on long-lived animals: Late stochastic and deterministic effects. 31st COSPAR Scientific Assembly, July 14-21, 1996, Birmingham, U.K.

Lett, J.T. Experimental models for cellular radiation targets: LET, RBE and radioprotectors. Adv. Space Res., 18(1/2), 31-40 (1996).

Lett, J.T. (abstract) Risks from space radiations: The need to determine damage to higher orders of DNA structure in mammalian chromatin. 31st COSPAR Scientific Assembly, July 14-21, 1996, Birmingham, U.K.

Lett, J.T. Radiation Research 1895-1995, Vol 2: Congress Lectures. Eds: Hagen, U., Jung, H., and Streffer, C. pp. 110-113 (1996). Proceedings of the 10th International Congress of Radiation Research, August-September 1995.

Lett, J.T. and Williams, G.R. (abstract) Effects of heavy ions on the DNA in the photorecetor cells of the rabbit: An overview of two decades of life space experiments. 31st COSPAR Scientific Assembly, July 14-21, 1996. Birmingham, U.K.

Williams, G.R. and Lett, J.T. Damage to the photoreceptor cells of the rabbit retina from ^{56}Fe ions: Effect of age at exposure, 1. Adv. Space Res., 18(1/2), 55-58 (1996).

Molecular Analysis of HZE Damage in Transgenic Mice

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Project Identification: 199-45-17-15
Initial Funding Date: 4/95
FY 1996 Funding: $115,955

Solicitation: 93-OLMSA-07
Expiration: 5/98
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 are using a transgenic mouse system that has been constructed so that every cell of the animal contains multiple copies of an integrated target gene. The use of this system will allow us 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 induced by HZE radiation, especially in nondividing tissues and germ and stem cells. The advantage of the transgenic mouse is that, in parallel with experiments using the integrated target gene, it will be possible to assess mutagenicity at another locus (hypoxanthine guanine phosphoribosyl transferase, hprt) and to use the micronucleus assay and chromosome painting to assess the frequency of cytogenetic damage induced in the bone marrow and peripheral blood erythrocytes of these mice. These last three end points can also be assayed in humans, using blood samples collected by venipuncture. This allows mutagenic and clastogenic endpoints to be correlated between the two species. We also propose using transgenic mice that have been crossed with mice which have either one or both copies of the p53 tumor suppressor gene inactivated. This gene is involved in responses to ionizing radiation, and the use of these mice should be informative for the mechanisms of radiation action. Mice that are hemizygous at the p53 locus should serve as models for individuals in the human population who may be carriers of recessive genes that predispose to cancer.

We have a transgenic mouse line established in our laboratory which has multiple copies of the bacterial lacZ gene integrated into every cell of the animal. We have also crossed these mice with those that have a targeted neo insertion into the Trp53 locus and so are nullizygous for p53 protein expression (supplied by Jackson Labs, Bar Harbor, ME). We exposed mice to iron ions (a significant component of the space radiation environment) and analyzed the induced mutational frequency and spectra in different tissues at sequential times following irradiation. We also examined the induction of chromosome aberrations using fluorescent *in situ* hybridization (FISH) with mouse-specific genomic probes (work performed in collaboration with Dr. J. D. Tucker, Lawrence

Livermore National Laboratory, CA) and mutation induction at the endogenous hprt locus (work performed in collaboration with Dr. V.E. Walker, Wadsworth Center, NY). We found that spontaneous mutant frequencies at the transgene (lacZ) locus are similar in liver and brain (4×10^{-5}) but are higher in spleen (5.5×10^{-5}). Mutation frequency is increased only slightly in liver following exposure to 1 Gy iron ions, while there is a significant increase in brain at early time points (1 week) which returns to background by 16 weeks following exposure. The response in spleen is complex and may reflect the diversity of cell populations which comprise this tissue. Although there was only a slight increase in mutant frequency in the liver following iron ion irradiation, molecular analysis of the recovered mutant transgenes revealed that there was an increase in the proportion of deletions recovered, and of these, the number that were very large deletions (i.e. extended beyond the transgene locus and into the flanking mouse sequences) was 5-fold higher than in mutants recovered from unirradiated animals.

Analysis of chromosomal aberrations by FISH in peripheral blood lymphocytes from exposed animals revealed a greater than 50-fold increase in translocations and a 10-fold increase in dicentrics at 1 week following exposure. By 16 weeks, the level of dicentrics had stabilized at 2-fold above background, and the translocations were still 5-fold above spontaneous levels. A notable finding was the extent and complexity of the damage induced by this form of ionizing radiation in the cells that were apparently hit. This could explain why there was little elevation in mutation frequency at the transgene locus in liver: cells that were hit by iron ions were so heavily damaged that they did not survive or underwent apoptosis, and so were not scored as mutants.

Mutation frequencies at the endogenous hprt locus were not elevated in animals exposed to the same dose of iron ion irradiation as in the above studies. This is surprising since cultured cells similarly exposed do have elevated mutation frequencies at this locus, and it suggests that there are additional surveillance mechanisms functioning in tissues for the removal of damaged cells that are not present *in vitro*. The similarity in the response at the hprt and lacZ loci validates our use of the transgene as a surrogate for endogenous genes. p53 nullizygous animals that were exposed at the same time had hprt mutant frequencies elevated 12-fold and the p53 heterozygotes had mutant frequencies elevated 7-fold. This suggests that tissues which do not have sufficient functional p53 protein to direct cells down an apoptotic pathway will have a higher proportion of damaged cells survive as mutants. We have also found elevated mutation frequencies at the lacZ transgene locus in p53 nullizygous mice following exposure to X rays. This is the first time that it has been demonstrated that loss of p53 function can induce changes in mutation frequencies.

These results indicate that exposure to iron ions causes extensive damage in cells that are traversed, that such cells appear to be readily removed from tissue populations in animals that have both copies of the p53 tumor suppressor gene intact, and that individuals (or cells) who have only one functional copy of the gene are at greater risk from such exposure. We plan to extend these studies to investigate the possible induction of genomic instability in different tissues of exposed transgenic animals that vary in their p53 status and genetic background.

This research is directed towards an understanding at the molecular level of the effects in humans of exposure to ionizing radiation. Although it is known that such exposure can cause life-shortening carcinogenesis, chromosome abnormalities, neurological damage, tissue damage, and cataractogenesis, the underlying molecular mechanisms for the induction and processing of this damage have not been well-characterized. An understanding of the basic molecular processes that are involved in the resolution of ionizing-radiation induced damage will enable greater accuracy in predicting the magnitude and nature of the response to ionizing radiation exposure in humans. It can also provide information on fundamental cellular processes such as the damage induction and response pathways, cell cycle controls, tissue-specific mutagenic mechanisms, and the induction of genomic instability. The assays that we are developing in these studies have the potential to provide a direct correlation between the damage induced by ionizing radiation, both on Earth and in space, in experimental animals and 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.

FY96 Publications, Presentations, and Other Accomplishments:

Lutze-Mann, L.H. (presentation) Transgenic Animals in Mutation Research (Satellite Meeting to the Environmental Mutagen Society Meeting), Sidney, B.C., Canada (March 20-23, 1996).

Lutze-Mann, L.H. (presentation) Environmental Mutagen Society Annual Meeting, Victoria, B.C., Canada (March 24-28, 1996).

Lutze-Mann, L.H. (presentation) Forty-Fourth Annual Meeting of the Radiation Research Society, Chicago, IL (April 14-17, 1996).

Lutze-Mann, L.H. (presentation) Seventh Annual Space Radiation Health Investigators' Workshop, Riverside, CA (May 14-17, 1996).

The Effect of Single Particle Traversals on a Mechanism of Cell-Cycle Regulation

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Funding:

Project Identification: 199-45-17-18
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $187,968
Students Funded Under Research: 2

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.

This project began as a study of cell-cycle regulatory responses to high LET particle traversal. Within the first six months of work, the research took on an entirely new direction when it was realized that we could visualize heavy particle traversals by assaying for *in situ* endonuclease activity, a DNA repair activity that probably linked to initiation of cell-cycle delay. We continue this work with major emphasis on DNA repair proteins.

Cellular Responses to One GeV/amu Iron Ion Exposure. Results of experiments done at the NASA BNL-2 run, held at the Brookhaven AGS in October of 1996, are beginning to yield interesting insights into DNA repair. Two types of cells were used, the cervical carcinoma cell line, HeLa, and the normal foreskin fibroblast cell strain, NFF. The doses used were calculated to give very low particle fluences to the cell nuclear cross-section, based on a track-averaged LET of 120 keV/μm and a HeLa cell nuclear cross-section of 125μm^2. For the one GeV/amu iron, four doses plus the sham control were used: 0 cGy = 0 particles/cell nuclear area; 9.6 cGy = 0.5 particles/nucl; 19.2 cGy = one particle/nucl; 38.5 cGy = two particles/nucl; and 76.9 cGy = four particles/nucl. After irradiation, cells were incubated for varying lengths of time, then fixed in one of three different fixatives and stored at 4 ^{0}C. In subsequent assays, the cellular DNA was probed by enzymatic addition of labeled dNTP's to 3'-OH ends, or probed for specific repair proteins by immunocytochemical methods. The cells were then viewed by confocal laser scanning microscopy to detect the location of the fluoresceinated probes.

Repair endonuclease activity. As in comparable alpha-particle experiments, the irradiated cells labeled in discreet, columnar foci when viewed in horizontal and vertical section. In contrast, labeling in the control cells were diffuse and spread across most of the cell nucleus. The analysis so far has indicated that 1) the cell receiving the lowest dose, 9.6 cGy, show labeled foci in a slim majority of the cells (this is the phenomenon we want to study); 2) higher doses show few if any foci, indicating that there are probably too many particle

traversals through the nucleus to allow for localized effects; and 3) foci appear in a few of the sham-irradiated cells. This third point raises the possibility that particles may be leaking past the beam plug, but it is more probable that the assay is showing a background with these cells. These data are nevertheless supportive of the hypothesis that during the two-hour incubation, repair endonucleases are recruited to these sites of highly localized DNA damage.

Repair protein induction. A set of normal human fibroblast NFF cell coverslips, exposed to either zero, one, two, or four particles/nucleus, then incubated for 3.5 hours before methanol fixation, were immunostained with antibody to MLH-1, a human protein implicated in mismatch repair. The control cells were not labeled, but for the irradiated cultures, the fraction of cells labeling in each were Poisson-distributed, and those cells that did label showed a dose-dependent intensity.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Metting, N.F. Alpha-particle exposure and the G2/M cell-cycle checkpoint in human cells. Seminar given at the Department of Radiation Oncology, Stanford University School of Medicine (April 3, 1996).

Metting, N.F. Progress on cell-cycle protein localization: Addition of DNA damage localization after alpha particle traversal. Seventh Annual Space Radiation Health Investigator's Workshop, Riverside, CA (May 14-17, 1996).

Metting, N.F. Visualization of alpha-particle traversals in cells. Seminar at the Center for Radiological Res., Columbia University, N.Y., NY (June 7, 1996).

Metting, N.F. and Braby, L.A. (Poster) Visualization of single Alpha particle tracks in DNA. Annual Meeting of the Radiation Research Society, Chicago, IL (April 14-17, 1996).

Metting, N.F. and Braby, L.A. Visualization of charged particle traversals in cells. 12th Symposium on Microdosimetry, Keble College, Oxford, UK (Sept. 29-Oct. 4, 1996).

Nelson, J.M., Brooks, A.L., Metting, N.F., Khan, M.A., Buschbom, R.L., Duncan, A., Miick, R., and Braby, L.A. Clastogenic effects of defined numbers of 3.2 MeV alpha particles on individual CHO-K1 cells. Radiat. Res., 145, 568-574 (1996).

Experimental Study of Nuclear Interactions Relevant to High Energy Heavy Ion Transport

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Funding:

Project Identification: 199-45-16-12
Initial Funding Date: 10/94
FY 1996 Funding: $529,000

Solicitation:
Expiration: 12/96
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, and to assist in the training of students and scientists new to the field of accelerator-based radiobiology. Fragmentation measurements are made by placing particle detectors in the path of beams of accelerated heavy ions which pass through biological samples, tissue-equivalent targets such as water or polyethylene, or shielding material such as aluminum. Radiation fields measured behind biological samples provide radiobiologists with a description of the radiation incident on their samples. This information can be important, as some degree of beam fragmentation is unavoidable in an accelerator experiment. Data on fragmentation in tissue and shielding are useful as a direct measure of the effects of the self-shielding of the human body and as input to and benchmarks of models of radiation transport. These models are an essential part of the solution to the space radiation problem, as it is impractical to empirically test the physical and biological effects of every possible combination of radiation environment and shielding material and thickness for every biological endpoint. The activities under this task include experiments at the Alternating Gradient Synchrotron (AGS) at Brookhaven National Laboratory (BNL) 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.

A second set of radiobiology and related physics experiments at the BNL AGS was completed, establishing the AGS radiobiology facility as a stable resource for heavy radiation experiments. Our group continued in support of NASA-sponsored radiobiology at the AGS and the LBL 88" cyclotron. Data taken at the AGS were compared with a new heavy ion fragmentation model developed at NASA LaRC. Also at the AGS detector, intercomparisons were carried out with an LET spectrometer which had been flown on the Mir space station, with CR-39 plastic track detectors and with a microdosimeter. The latter was done in collaboration with the LBL-CSU NSCORT in Radiation Health.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Zeitlin, C., Heilbronn, L., Miller, J., Frankel, K., Gong, W., and Schimmerling, W. The fragmentation of 510 AMeV ^{56}Fe in Polyethylene. Radiat. Res., 145, 666 (1996).

Zeitlin, C., Heilbronn, L., Miller, J., Schimmerling, W., Townsend, L.W., and Wilson, J.W. The fragmentation of 510 AMeV ^{56}Fe in polyethylene II. Comparisons between data and a model calculation. Radiat. Res., 145, 655 (1996).

3D ORAM Dosimeter for Space Radiation Environments

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Funding:

Project Identification: 106-20-01-05

Solicitation: 93-OLMSA-07

Initial Funding Date: 2/95

Expiration: 2/98

FY 1996 Funding: $85,465

Students Funded Under Research: 2

Joint Agency Participation: DoE

Task Description:

Our objectives are to develop a crew dosimeter for heavy-charged-particle (HZE) monitoring, applicable to the U.S. space flight program. The dosimeter will enable personnel dosimetry in space radiation environments, providing radiation protection to humans in space. The use of radiation-induced changes in three-dimensional optical random access memories (3-D ORAM) provides the basis for the approach. ORAM is a small cube (a few 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.

The original direction of the task was limited to theoretical studies to determine the effects of HZE particles on 3-D ORAM. Recently we discovered an experimental method to measure these radiation effects using a laser scanning confocal microscope (LSCM). The direction of the task was therefore updated to include experimental investigation of the radiation effects on 3-D ORAM.

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 continued our theoretical work to simulate the radiation effects on three-dimensional computer optical memories. In addition, we started a series of experiments in an effort to actually measure these radiation effects using a laser scanning confocal microscope (LSCM). The ORAM material used was spyrobenzopyran-doped poly(methyl methacrylate) (SP/PMMA.) Irradiations were performed using two MeV alpha particles from the tandem accelerator facility at the Naval Surface Warfare Center, White Oak, MD. Our preliminary results show measurable radiation effects for relatively high particle fluence of approximately 10^{11} particles/cm^2. Future plans are to obtain thin films of SP/PMMA and improve our measurement sensitivity. We hope to be able to extend our measurements to lower values of particle fluence, by using near field scanning optical microscopy techniques (NSOM).

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.

FY96 Publications, Presentations, and Other Accomplishments:

Emfietzoglou, D. and Moscovitch, M. Theoretical basis for solid state microdosimetry using photochromic alterations. Proc. 12th Symposium on Microdosimetry, Oxford, U.K. (September 1996).

Patent Approved, U.S. Patent #: 5,498,876 Moscovitch, M. "Neutron spectrometer, real-time dosimeter and methodology using three-dimensional optical memory."

Moscovitch M. and Emfietzoglou, D. Simulation of radiation effects on three-dimensional computer optical memories. J. Appl. Phys., Manuscript No. JR-5080, (in press).

Radiation and Environmental Health

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Funding:

Project Identification: 199-45-14-11
Initial Funding Date: 10/94
FY 1996 Funding: $300,000

Solicitation:
Expiration: 12/97
Students Funded Under Research: 0

Task Description:

No additional information was supplied by the principal investigator.

Effects of Exposure to Heavy Particles

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Funding:

Project Identification: 199-45-17-16
Initial Funding Date: 2/95
FY 1996 Funding: $32,031

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 1

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 one GeV/n iron (^{56}Fe) particles on: 1) dopamine-mediated motor behavior, by studying upper body strength measured by a wire suspension task, 2) oxotremorine enhanced dopamine release from striatal tissue, using HPLC, and 3) the behavioral toxicity of one GeV/n iron particles, measured using the conditioned taste aversion paradigm. These experiments were designed to 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 one GeV/n iron-56 (^{56}Fe) particles and to examine the range of dopamine mediated behaviors that may be affected by exposure to these iron particles.

The goals of the current experiments were: 1) to complete the comparison of the effects of exposure to one GeV/n ^{56}Fe particles to the effects observed following exposure to 600 MeV/n ^{56}Fe particles obtained using the BEVALAC at LBL; and 2) to begin a series of experiments designed to advance our understanding of the mechanisms by which exposure to heavy particles can produce changes in neurobiological and behavioral functioning. The specific endpoints were to determine: (1) whether exposing rats to one GeV/n ^{56}Fe particles dopamine-mediated behaviors in addition to motor behavior; (2) whether or not heavy particle-induced changes in signal transduction could be reversed by increasing the concentration of Mg^{2+} in the perifusion medium; and (3) the role of changes in membrane fluidity are involved in mediating heavy particle- induced changes in signal transduction.

Because an amphetamine-induced taste aversion, unlike one produced by lithium chloride, is dependent upon the integrity of the dopaminergic system, the disruption of an amphetamine induced aversion would support the hypothesis that exposure to ^{56}Fe particles could disrupt a range of behaviors mediated by dopamine in addition to motor behavior. Exposing rats to one Gy, but not 0.5 Gy, of one GeV/n ^{56}Fe particles prevented the acquisition of an amphetamine-induced aversion. Neither dose affected the acquisition of a lithium chloride-induced aversion. Although these results are similar to those obtained with 600 MeV/n ^{56}Fe particles, the lower energy particles were significantly more effective, producing an effect at a dose of 0.1Gy.

This experiment examined whether increasing Mg^{2+} concentration of the basal release and depolarization media from one to two mM would reverse the deficits in dopaminergic function in striatal tissue in irradiated rats, as it does in aged rats. Compared to non-irradiated controls, rats exposed to one Gy of one GeV ^{56}Fe particles showed a significant decrease in oxotremorine enhanced dopamine release (104.73 ± 5.94, 56.19 ± 3.57 pmoles/mg protein, respectively). Increasing the concentration of Mg^{2+} in the perifusion medium of irradiated animals produced a significant increase in dopamine release (99.17 ± 13.44 pmoles/mg protein). The observation that it appears equally effective in reducing the signal transduction deficits in aging animals and in animals exposed to heavy particles supports the hypothesis that similar mechanisms mediatie the alterations in dopaminergic function.

Research in aged animals suggests that at least part of the alterations in mAChR-G protein coupling observed in irradiated rats may be the result of structural changes in the membrane, possibly changes in membrane fluidity. A preliminary experiment conducted during this visit to BNL indicated that, just as was seen in old animals, incubating tissue of rats exposed to one Gy of one GeV/n ^{56}Fe particles in s-adenosyl methionine enhanced K^+-evoked dopamine release. However, an increase the sample size and in the dose (to 2.5 Gy) is necessary to confirm these findings.

Continuing experiments are designed to advance our knowledge of the range of behaviors affected by exposure to heavy particles and our understanding of the mechanisms by which exposure to heavy particles can produce changes in neurobiological and behavioral functioning. These experiments will involve: 1) the determination of whether there is recovery of function in dopamine-mediated taste aversion learning following exposure to ^{56}Fe particles by testing rats for an amphetamine-induced taste aversion 60 days following irradiation; 2) further evaluation of the role of particle-induced changes in membrane fluidity in producing deficits in dopaminergic function; 3) an initial evaluation of possible relationships between the age of the organism and vulnerability to the neurochemical effects of exposure to heavy particles by studying these processes in young and old rats; and 4) an initial study the effects of exposure to ^{56}Fe particles on the morphology of the brain using immunohistochemical procedures.

The research which we have conducted previously has shown that exposing young rats (less than 3 mo. old) to low doses of heavy particles (^{56}Fe, 600 MeV/n) has shown that the neurochemical deficits produced by this exposure are similar to those that are observed in aged rats (24 mo. old). The research which we have just completed at BNL indicates that similar deficits are observed following exposure to one GeV/n ^{56}Fe particles as well. Thus, exposing rats to low doses of ^{56}Fe particles provides a way to accelerate aging in experimental animals with respect to certain brain and behavioral parameters so that experimenters do 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.

FY96 Publications, Presentations, and Other Accomplishments:

Joseph, J.A., Erat, S., and Rabin, B.M. Cosmic radiation (^{56}Fe Particles) effects on signal transduction in the CNS: Implications for immediate or delayed motor and cognitive deficits as a function of age. 7th Annual Space Radiation Health Investigators' Workshop. Riverside, CA (May 14-17, 1996).

Joseph, J.A., Erat, S., and Rabin, B.M. (abstract) Selective efficacy of space-like radiation effects (56Fe particles) on muscarinic neurotransmitter sensitivity and motor behavior. 31st Scientific Assembly, Committee on Space Research (COSPAR), Birmingham, England, p. 331 (July 14-21, 1996).

Rabin, B.M. and Joseph, J.A. Relationship between the behavioral toxicity of 56Fe particles and LET. 7th Annual Space Radiation Health Investigators' Workshop. Riverside, CA (May 14-17,1996).

Rabin, B.M., Joseph, J.A., and Erat, S. Effects of exposure to different types of radiation on behaviors mediated by peripheral or central systems. 31st Scientific Assembly, Committee on Space Research (COSPAR), Birmingham, England, p. 331 (July 14-21,1996).

Cooperative Radiation Research (NCI)

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Funding:

Project Identification: 199-08-17-64
Solicitation:

Initial Funding Date: 2/95
Expiration: 2/00

FY 1996 Funding: $
Students Funded Under Research: 0

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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. Two researchers are associated with this joint effort which will end in FY97.

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.

Project II Plateau-phase fibroblasts were exposed to 0.22, 0.34, 0.43, 1.0, 5.9, and 13.6 MeV monoenergetic neutrons and to low dose-rate Cs-137 gamma rays. All categories of asymmetric chromosomal changes were recorded with linear dose-response relationships throughout. The highest RBE (24.3) for 0.22 and 0.43 MeV neutrons was observed for intrachromosomal deletions, a category of chromosomal change common in solid tumors. These studies indicate that low-energy neutrons close to the border of where the radiation-weighting factor changes from 20 to 10 are very effective at inducing interstitial deletions. Confirmation of these findings and extension to lower energies is highly desirable.

Project III Optimal sensitivity for neutron-induced transformation (5.9 MeV; single dose of 0.6 Gy) of C3H10T1/2 mouse cells was found in the G1 phase of the cell cycle compared to x-ray-induced transformation which localized in the G2 phase of the cell cycle. In a second component, human papillomavirus-immortalized human bronchial epithelial cells irradiated with a single 30 cGY dose of HZE Fe ions became tumorigenic 3-4 months post irradiation at frequencies estimated to be ~4 x 10^{-7}. Molecular analysis of tumorigenic cell lines revealed defects associated with the G1/S checkpoint (e.g., over expression of cyclin D1). This model provides a unique opportunity to study cellular and molecular changes at various stages of radiation-induced cancer.

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.

Syngeneic human B-lymphoid cell lines (TK6, WTK1) irradiated with low fluences of HZE (1090 MeV) Fe ions demonstrated high levels of programmed cell death by apoptosis. Apoptosis was suppressed in cells that over expressed the proto-oncogene bcl-2 or the defective bcl-XL. This is the first demonstration of apoptosis induction by low fluences of HZE Fe ions. Over-expression of the bcl-2 or the aberrant bcl-XL result in accumulation of mutations among progeny of irradiated cells and may lead to expression of genomic instability.

There are approximately one million new cancer cases every year, of which approximately half receive radiotherapy either alone or in combination with other treatment. Major advances in diagnosis and treatment have been made in recent years including 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.

HZE Radiation Genotoxicity in Cultured Mammalian Cells

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Funding:

Project Identification: 199-45-17-24
Solicitation: 95-OLMSA-01
Initial Funding Date: 12/95
Expiration: 11/96
FY 1996 Funding: $ 129,794
Students Funded Under Research: 10

Task Description:

Sojourners in deep space will be exposed to high energy (HZE) ionizing radiations. The evidence now available is that these radiations potently induce genotoxic effects like cell death, mutation, and chromosomal aberrations. The last two effects are particularly worrisome since mutated cells are known to cause cancer. However, a much better understanding of the mutagenic potential of HZE irradiations is essential to making rationale decisions about risks to space travelers. Our participation in a NASA NSCORT gave us access to HZE ^{56}Fe at LBL where some data were generated for the genotoxicity in the A_L human x hamster cell hybrid cell line which is, per unit dose, more sensitive to HZE- mutagenesis than any other *in vitro* cell line. Genotoxicity can, therefore, be studied at relatively low doses. But our resources via the NSCORT, while appreciated greatly, are quite limited. Additional funds have been requested to speed up and expand the work with HZE irradiations, using principally ^{56}Fe, 150 Gev/m at BNL. The A_L human x hybrid *in vitro* mutation assay will be employed to quantify HZE mutagenic activity and to identify enough mutants to be subjected to Southern and PCR analysis to define mutational spectra for low doses of HZE irradiations. The program would be expanded to include extensive molecular cytogenetic analysis of chromosomes of the A_L hybrid and of normal human fibroblasts. These data on chromosomal mutation and chromosomal aberrations are not now available and would help in understanding and predicting radiation risks of space travel.

The rationale for our work is that mutation drives genetic diseases including cancer so that studies of mutagenesis in cultured cells can shed light on risks of cancer, and illuminate mechanisms of carcinogenesis. Excellent progress has been made on the task of evaluating the mutagenic potency to mammalian cells (A_L human-hamster hybrids) of some of the radiations (HZE-Fe, nitrogen, protons, ^{137}Cs-γ) to which travelers in deep space will be exposed. We find that per unit dose (Gy) HZE-Fe and nitrogen atoms are about 50% more mutagenic than ^{137}Cs-γ (used as a control); protons are about 50% less mutagenic. But when mutant yields are expressed as mutants per surviving clonable cell, or per mean lethal dose (D_0), HZE-Fe, nitrogen, and protons are actually less mutagenic, by about half, than ^{137}Cs-γ rays. As we have pointed out, it is this latter measure,

mutants per surviving cell, that is more relevant to carcinogenesis than mutants per unit dose. On the other hand, although the quantity of mutants per survivor is less for HZE, nitrogen, and protons than for ^{137}Cs-γ, more of the mutants induced by these three test radiations are of the complex and apparently unstable kinds that have been particularly implicated in carcinogenesis. These initial analyses indicate that HZE-Fe, nitrogen, and protons induce fewer but more dangerous kinds of mutants than ^{137}Cs-γ rays. We are applying Southern, polymerase chain reaction (PCR) and molecular cytogenetic analyses to mutants derived from standard A_L cells treated with a variety of radiation and exposure regimens to test this important hypothesis. We have also begun studies with higher LET radiations of the phenomenon of adaptive response where a low dose of low LET radiation (^{137}Cs-γ, 3 cGy) substantially reduced the number but increased the proportion of complex mutants induced by a later, higher dose. The sensitivity of standard A_L cells and our new hybrids such as A_LC to mutation is also allowing us to evaluate if and how radioprotective drugs such as WR-1065 and the thiazolidine prodrugs of cysteamine and cysteine affect the mutant quantity and quality of space radiations. The rationale for these studies is that an ounce of prevention is truly worth a pound of cure, or in this case an ounce of chemical may be worth several pounds of shielding. The emphasis will be on understanding what genetic (DNA repair, ploidy, linkage) and radiologic factors (radiation quality, dose rate, accompanying drug treatment, etc.) govern induction of these complex, unstable mutants that appear to be especially linked to carcinogenesis.

The goal of our NASA-sponsored program is to provide data that will improve predictions of risk and reduce the incidence of radiation-induced genetic disease such as cancer in space travelers. We are using our sensitive, relevant, but relatively cheap mutations assays to provide data on risks of genetic diseases associated with exposures to radiation, or to any other DNA damaging agent that exists in the environment in space or on earth. These studies are shedding light on mechanisms of mutagenesis and relationships of mutation to carcinogenesis. The sensitivity to mutation of A_L-based assays is allowing us to investigate effects of potential antimutagens/anticarcinogens at the low doses of mutagen where most human exposures occur using low, non toxic doses of antimutagen.

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High-resolution Digital Mammography/NCI

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Project Identification: 199-45-17-22
Initial Funding Date: 01/95
FY 1996 Funding: $250,000
Joint Agency Participation: NIH

Solicitation:
Expiration: 12/97
Students Funded Under Research: 5

Task Description:

The specific aims of this project are focused on the development, optimization, and pre-clinical evaluation of a scanning slot x-ray detector for digital mammography. The technical objective is to achieve the following imaging characteristics of the scanning slot x-ray detector: (1) >80% quantum absorption efficiency; (2) 15 lp/mm limiting spatial resolution; (3) ~0.5% contrast sensitivity; (4) >70% zero spatial frequency detective quantum efficiency (DQE(0)); (5) >400:1 dynamic range; and (6) imaging a 20 cm x 24 cm breast in ~four seconds.

The primary goal is to develop a plastic scintillating fiber screen (SFS) based scanning slot x-ray detector for digital mammography. The detector will be made of eight SFS-image guide-CCD modules, which form a total detector cross sectional area of 0.8 cm x 20 cm. The 2 cm thickness SFS is made of 20 mm diameter plastic scintillating fibers. The image guide has an input to output ratio of 1:1. Pixel charges from all the CCDs (TDI mode) will be read out in parallel and digitized to 14 bit. An on-line flat-fielding unit will be implemented to correct detector non-uniformity. The final image format will be ~ 8K x 8K. Current status of this project: (1) Three key advancements over conventional plastic scintillating fiber technology have been developed to maximize scintillating output from the SFS: a) improved scintillating fiber energy conversion efficiency from 3% to >4.5%; b) increased scintillating fiber light collection efficiency from 3% to 7.5%; and c) loaded 7.5% by weight tin in plastic scintillating fiber core material; (2) Plastic scintillating fibers with the above characteristics have been produced. Intense effort is now devoted to improve the fiber production environment and techniques to produce clean fibers of uniform diameter to enhance light transmission; (3) Techniques to make the parallax corrected SFS and continuous image guide module have been developed; and (4) A prototype scanning imaging system has been integrated and tested. This system includes a dedicated mammography x-ray unit, an TDI-CCD camera, a linear scan table, and an PC-based frame grabber.

The second effort will be to compare the use of SFS with the use of a new CsI:Tl screen in a slot x-ray detector. The purpose is to optimize the x-ray detection material to develop a scanning slot x-ray detector with the best possible imaging performance for mammography. This new prismatic type CsI:Tl screen has both high spatial resolution and x-ray absorption efficiency. Its scintillation light emission spectrum matches well with the CCD spectrum response. Its scintillation decay time is only ~1 ms which eliminates the afterglow effect associated with the use of a rare-earth phosphor screen in a scanning slot detector. Our preliminary tests of this CsI:Tl screen have shown very encouraging results. In this research, clear plastic optical fiber image guides with 1:1

input/output ratio will be developed to couple the CsI:Tl screen to the CCDs. With this configuration, it is possible to construct a high quality digital mammography system which will be very competitive with the other technologies using expensive glass fiber image guides. The performances of the SFS-based and CsI:Tl screen-based scanning slot x-ray detectors will be compared by measuring the detector contrast sensitivity, modulation transfer function (MTF), noise power spectrum (NPS), and DQE. Phantom tests will be performed to detect the detector nonuniformity and artifacts.

The final goal is to perform comparative receiver operating characteristics (ROC) analysis to evaluate the performance of the optimized scanning slot x-ray detector using an anthropomorphic breast phantom. Simulated features (masses and microcalcifications) will be randomly positioned on the breast phantom to produce different image backgrounds around the simulated features. Radiologists' performance to detect of the presence of masses and microcalcifications will be evaluated to compare the digital system and a dedicated screen-film system.

To summarize our progress after two years of work, we have:

(1) successfully developed one plastic scintillating fiber slot module (SFS + 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 specially-developed scintillating dye which has a measured energy conversion efficiency of 4.5% from x-ray energy to visible photon energy, a 1.5 times improvement compared to most plastic scintillators. Experiments to measure the SFS scintillation light output and resolution are being conducted.

These achievements are critical in realizing the goals of this research. The amount of scintillation light output from SFS 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 lp/mm 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 a very short time. The success in setting up these components allows the prototype SFS 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 two 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 two MHz readout rate and 25°C.

(4) investigated the effect of the scattered radiation in a scanning slot imaging system using Monte Carlo methods. The results show that sufficient scatter rejection can be achieved using an airgap method with negligible dose penalty.

(5) explored the use of a new CsI:Tl screen as an alternative to the use of an SFS in a scanning slot x-ray detector for mammography. The preliminary results obtained are very encouraging.

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 mammographic systems. Due to the very high spatial resolution and contrast sensitivity, the camera will be significantly more sensitive to the earliest signs of the disease.

This technology for digital radiology can be easily extended to general radiology for the chest and major organs. In this case, the typical x-ray energies are increased from about 20 keV to 80 keV. The real-time nature of image acquisition and display is particularly important for trauma or battlefield patients.

There are major applications of large area, high resolution, real time digital radiographic cameras for industrial, aeronautical, and space applications. High performance, composite materials are increasingly being used in these industries. This technology can meet the required specifications to optimize the processing and perform quality control of components made of these new materials.

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Radiation Anticarcinogenesis by Thiazolidine Prodrugs

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Project Identification: 199-45-17-23
Initial Funding Date: 3/96
FY 1996 Funding: $170,641

Solicitation: 95-OLMSA
Expiration: 2/99
Students Funded Under Research: 1

Task Description:

The long-term objective of the research is to develop nontoxic, radiation protectors which, when taken orally, will diminish the long-term genetic consequences of space radiation exposure. Available compounds which serve as effective radioprotectors cause nausea when taken orally and are cytotoxic at radioprotective concentrations. A class of thiazolidine radioprotectors has been developed at the University of Utah. These compounds are nontoxic at radioprotective concentrations. The specific aims of this proposal are designed to determine the capacity of these thiazolidine prodrugs to protect mammalian cells from the long-term genetic effects (e.g., mutagenicity and carcinogenicity) which result from exposure to space radiations. Mammalian cells (mouse 10T1/2 and human mammary epithelial cells) will be exposed to g-ray (^{137}Cs) or proton irradiation in the presence and absence of thiazolidine prodrugs. Alternatively, previously irradiated cells will be exposed to thiazolidine prodrugs up to 24 hours after irradiation. The capacity of these compounds (present during or after irradiation) to protect mammalian cells from the induction of cytotoxicity (measured as single cell survival), chromosome damage (translocations measured by fluorescence *in situ* hybridization of mitotic chromosomes with chromosome specific DNA probes), mutations (*hprt* locus mutagenesis measured as the production of 6-thioguanine resistance), and carcinogenesis (measured as induction of cell transformation) will be quantified.

We expect to identify thiazolidine prodrug concentrations which, when administered during or after irradiation, protect mammalian cells from the induction of deleterious genetic effects such as carcinogenesis. The future goals of this research will be to determine the utility of these compounds for animal radioprotection, and the potential for oral administration of these compounds to humans. If these compounds are found to be nontoxic at effective radioprotective concentrations, they will be indicated as useful radioprotectors for astronauts.

The overall goal of this proposal is to determine whether thiazolidine prodrugs are useful for reducing radiation (in particular gamma and proton)-induced mutagenesis and carcinogenesis. Our first year's aims were: 1) to determine thiolamine-induced, human mammary epithelial (HME) cell toxicity, if any, and 2) develop dose-effect relationships for radiation-induced cytotoxicity and thiolamine radioprotection capacity. Before initiating these studies we needed to establish an appropriate cell line for the proposed work. The 184A1 HME cell line is an immortal cell line which exhibits a non-transformed phenotype (i.e, is contact inhibited and does not form tumors in mice) and exhibits a high plating efficiency (typically greater than 60% in our hands). The 184A1 cell line obtained was aneuploid (approximately 40% tetraploid), and a diploid cell line (184A1C1) maintaining a stable ploidy for up to 20 passages was cloned.

^{137}Cs-irradiated 184A1C1 cells exhibit a toxicity curve with a D_q of 0.4 Gy and a D_0 of 1.8 Gy. The radiation sensitivity of these cells is equivalent for both proliferating and confluent (non-growing) cells assayed either immediately or 24 hours after irradiation. Thus 184A1C1 cells exhibit little or no potentially lethal cell recovery during post-irradiation incubation. 184A1C1 cells exhibit a background mutation frequency of approximately 3 x 10^{-5} (about 30 mutations per 10^6 viable cells plated) at the X-linked *hprt* locus; a value comparable to values for comparable cell lines. We are presently developing methods to quantitate radiation-induced *hprt* mutation induction and chromosome translocations in 184A1C1 cells.

The sensitivity of 184A1C1 cells to thiolamines is cell growth state dependent. WR1065 (a conventional thiolamine radiation protector) exposure for 24 hours is extremely cytotoxic unless it is thoroughly washed away from the cells. In "washed" cells, the cytotoxicity of 4 mM WR1065 for 24 hours is 50%. In proliferating cells, exposure to 5 mM Ribose Cysteine (a thiazolidine prodrug) for 24 hours causes 50% cytotoxicity while a similar treatment produces no cytotoxicity in confluent cells. Exposure to 4 mM WR1065 or 10 mM cysteine for 60 minutes at 37°C reduces radiation-induced DNA strand breakage 60 and 40%, respectively. Exposure to 10 mM RibCys for 3 hours reduces radiation DNA strand breakage by 10%.

The primary goal of this research project is to develop nontoxic protectors which, when taken orally, will diminish the long-term genetic consequences of space radiation exposure. A natural extension of this research will be the utilization of compounds found to be effective protectors for reducing the genetic risk in humans exposed routinely or accidentally to ionizing radiations in the earth environment. Nontoxic radioprotective compounds will be useful for reducing tissue toxicity and carcinogenesis in individuals exposed to ionizing radiation in their work environment. Additionally, nontoxic, radioprotective compounds will be useful for normal tissue protection during cancer radiation therapy, decreasing the risk and increasing the effectiveness of cancer radiotherapy.

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Space Radiation Transport and Interaction

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Project Identification: 199-45-16-11
Initial Funding Date: 10/94
FY 1996 Funding: $222,000

Solicitation:
Expiration: 10/97
Students Funded Under Research: 7

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 were recently adopted by the Naval Research Laboratory for use in the Space Environment and Effects Project). Although great uncertainties remain in the methods and database, they are accepted as the best available and illustrates the potential impact of the current studies on space and aircraft technology. Near term activity consists in re-evaluation of nuclear absorption and atomic cross section databases according to recent experimental data; re-evaluation of media modified two-body interaction amplitudes; development of nuclear cluster models for improved fragmentation dynamics (collaboration with workers at the new DoE/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 (199-45-16-12); development of higher order neutron propagators; determine effects of current biophysical models on shield characterization; examine dose rate effects in solar flare exposures (collaboration with Oak Ridge National Lab.); examine effects of G1 and G2 blocking kinetics, cellular repair kinetics, and signal transduction (collaboration with Johns Hopkins Oncology Center); and develop analysis programs for space flight validation of environmental models, transport codes, and anatomical models (collaboration with JSC).

A high charge and energy (HZE) ion transport code HZETRN 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 latest version of the HZETRN code uses the updated NUCFRG2 code as the nuclear fragmentation database. 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. 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. 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. A new version of nuclear de-excitation based on master equations has shown great promise in evaluation of the final fragment distribution as a result of the simplifying form of the emission spectrum at high excitation energies and has resulted in a new database with good agreement with the recent experimental data measured at the AGS by Jack Miller's group using the 1.05 GeV/n iron beam. A reevaluation of the atomic and nuclear absorption cross sections have been completed with great improvements in our nuclear absorption database. In collaboration with Johns Hopkins University, we have developed a theoretical description in a mathematical frame work for gene expression related to DNA damage. We apply this model to the inhibition of the kinase activity of complexes of cyclins D and E with cyclin dependent kinases (cdk) through increases in mRNA levels of the cyclin kinase inhibitor, p21. The model developed is in agreement with existing experimental data for normal and tumor cells *in vitro*. We have also examined the timing of radiation insult and resulting cell-cycle arrest in G1 phase and the role of p53 and pRb status.

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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Funding:

Project Identification: 199-45-17-11
Initial Funding Date: 9/91
FY 1996 Funding: $0
Joint Agency Participation: DoD

Solicitation:
Expiration: 3/95
Students Funded Under Research: 0

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

This project was begun with support of NASA Life Sciences Grant NAG-9-528, and continues under Grant NAGW-3912. 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 was suspended pending the award of the funds to cover the work proposed in the second 12-month performance period of NAGW 3912. This work 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. On January 29,1996, NAGW-3912 Supplement 2 awarded the Grantee $55,400, for a period of 18 months beginning on April 1, 1995. Work was

resumed on the project in February 1996, and all tissue processing was completed in September 1996. As of February 10, 1997 the tissues are in the process of evaluation by an independent pathologist who is documenting all relevant radiation related lesions. A no-cost extension of the performance period was granted in February 1997 for the purpose of allowing sufficient time for interpretation of results, statistical analysis and final report preparation. The expected completion date is June 30, 1997.

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.

Neoplastic Cell Transformation With Protons and HZE

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Project Identification: 199-45-11-66
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $203,000
Students Funded Under Research: 2

Responsible NASA Center: JSC

Task Description:

The energetic electrons, protons, and heavy ions that constitute space radiation are hazardous to human health. High-LET heavy ions are particularly effective in causing various biological effects, including cell inactivation, mutation, and cancer. Among these biological effects, the induction of neoplasm is the most important late effect to be considered in radiation risk assessment because astronauts usually are chronically exposed to low doses. During a long-term space flight, such as a mission to Mars, astronauts will be exposed to considerable amounts of galactic cosmic rays (GCR). To ensure proper protection of astronauts and the success of a long-term mission, a better understanding of the carcinogenic effects of energetic protons and heavy ions is most essential. The major objectives of this proposal are to quantitatively measure the oncogenic effects of energetic protons with tissue-equivalent material shielding, to determine the relative biological effectiveness (RBE) of high-energy heavy ions, to gain a better understanding of the kinetics of repair of oncogenic damage, to examine the carcinogenic effect of gamma rays at very low dose rates, and to characterize the changes of growth properties and karyotype of radiation-transformed cells. We will show: 1) that high-energy heavy ions (>1GeV/u) are effective in causing neoplastic transformation of mammalian cells and in producing irreparable oncogenic lesions; 2) that the effectiveness of protons in causing cell transformation will be altered by tissue-equivalent material shielding; and 3) that the dose rate reduction factor for very low dose rate gamma rays can be more than three in confluent (G1) cells. To accomplish these objectives, we will conduct proton experiments using the synchrotron at the Loma Linda Medical Center in California during the first and second year, high-energy heavy-ion studies at AGS of Brookhaven National Laboratory in the second and third year, and low-dose-rate experiments with the gamma-ray source at NASA Johnson Space Center when proton and ion beams are not available. Experimental results so obtained should significantly increase our knowledge of the oncogenic effects of space radiation and should help to reduce the large uncertainty presently existing in radiation risk assessment.

These research studies are specifically designed to test the hypotheses that galactic cosmic radiation increases cell inactivation and that chromosomal aberrations 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, cytogentic 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, cytogenetic 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, the experiments done were mainly on collecting quantitative and qualitative information on cell killing, chromosomal aberrations, and neoplastic cell transformation by charged particles with and without shielding using preliminary data obtained last year to design these experiments. Molecular studies with human cells transformed by heavy ions were continued.

We have conducted experiments with 250 MeV protons at Loma Linda University Medical Center for survival and chromosomal aberrations determination. The kinetics of chromatin damage repair, formation of various types of chromosomal aberrations, including translocation, dicentrics, etc., in Go/G1 human cells were determined with fluorescence *in situ* hybridization (FISH) and premature chromosomal condensation (PCC) techniques. Preliminary results showed that the total number of fragments decreased with incubation time, suggesting rejoining of chromatin breaks. The translocation and dicentrics, however, increased with time and reached a plateau about 10 hr post irradiation. For survival and cytogenetic damages, the RBE value was about 1.0.

A better understanding of both the acute and the late effects of high-LET charged particles is essential for assessing radiation risk to astronauts for long-term space exploration missions. We conducted cell experiments with 1 GeV/u iron particles accelerated at AGS of Brookhaven National Laboratory in 1996 and have obtained some preliminary results on lethal and cytogenetic effects of these particles in mammalian cells. Cell killing can lead to functional alterations in tissues and cytogenetic damages can cause mutation and oncogenic transformation. We irradiated normal human lymphocytes and mouse embryonic fibroblasts (C3H10T1/2) *in vitro* to determine the dose-response relationships for chromosomal aberrations and cell killing, respectively. We used fluorescent FISH techniques for chromosomal aberrations analysis and FISH and premature chromosome condensation (PCC) to measure the kinetics of rejoining and misrejoining of chromatin breaks. Preliminary results showed that the dose-response relationship is linear and about the same for initial induction of PCC fragments in human lymphocytes exposed to gamma rays, protons or iron particles. The kinetics of rejoining of PCC fragments, however, was different for different types of radiation. Less than 10% of initial number of fragments, for example, could be observed in cells exposed to protons or gamma rays after 10 hr incubation at 37°C. About 50% of PCC fragments, on the contrary, were found in cells exposed to iron particles at 10 hr post irradiation. While the total amount of PCC fragments decreased with incubation time, the frequency of complex exchanges and total exchanges per cell increased exponentially and reached a maximum at about 10 hr. Interestingly, there was no significant difference in kinetics for exchange formation induced by protons, iron particles, or gamma rays. Our results also showed that the RBE for different PCC chromosomal aberrations was not the same—highest for reciprocal exchanges and lowest for complex exchanges. Our survival studies indicated an exponential dose-response curve for mouse embryonic fibroblasts (C3H10T1/2) exposed to iron particles with 0- or 5-cm tissue equivalent material shielding. The survival curve for 10T1/2 cells, however, showed an increase of survival and a small shoulder when the plating was delayed for abour two days. Furthermore, the cells with 5-cm tissue equivalent material shielding showed significantly higher survival rate than that without shielding, suggesting fragmented iron particles can be less effective in causing cellular damages. These findings are in consistent with our hypotheses and suggest further studies.

Molecular analysis of cancer genes in human mammary epithelial cells transformed by heavy ions was continued. The expression of both p53 and Rb genes was determined and no difference was found from that in control cells.

In addition to above studies, we recently performed cell experiments with carbon and neon ions generated at the Heavy-Ion-Medical Accelerator, in collaboration with scientists at the National Institutue of Radiological Sciences (NIRS) in Chiba, Japan and irradiated samples are under analysis.

This research work seeks to understand the carcinogenic effects of low- and high-LET radiation in mammalian cells. Radiation is part of our environment and can cause genetic alterations and cancers in humans. On the other hand, with proper control, ionizing radiation, including x-rays, g-rays, neutrons, and charged particles, can be useful for treating various human diseases. In fact, cancer radiotherapy with protons and charged particles is either in development or in practice in USA, Asia, and Europe. Our research studies on the effectiveness of low-LET radiation at low dose rates in causing oncogenic cell transformation will add quantitative information to the existing data for assessing radiation risk to ground radiation workers. Data from our high-LET radiation studies will be valuable for understanding the health effects of neutrons to radiation workers of nuclear reactors and for estimating the cancer risk of radon gas to the general public. The shielding studies with protons and carbon ions provide useful information for making treatment plans, since tissue-equivalent materials and the human body are part of the necessary shielding during radiotherapy. The cell fusion experiment, cancer genes studies, and the analyses of chromosomal aberrations in non-transformed and tumorigenic cells shed light on the basic mechanism(s) of carcinogenesis by radiation.

FY96 Publications, Presentations, and Other Accomplishments:

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Intestinal Adaptation in Microgravity Drug and Nutrient Adsorption

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Funding:

Project Identification: 199-18-17-20
Solicitation: 95-OLMSA-01
Initial Funding Date: 12/95
Expiration: 11/97
FY 1996 Funding: $99,746
Students Funded Under Research: 2

Task Description:

The physiologic changes which occur under microgravity conditions, can have a significant impact on drug and nutrient absorption, metabolism and utilization. It is the long term goal of this research to quantitate the alterations of gastric emptying, intestinal transit and absorption, and changes in metabolic pathways in a microgravity environment.

Revised Application: This proposal is a revised application based on an application submitted in 1993 for the Ground Based and Small Payloads Research in Life Sciences, NASA Research Announcement NRA 93-0LMSA-07. The current application has been revised based on the previous technical review. The major short coming noted in the previous review was the lack of preliminary results in normal human subjects in order to validate the *in vivo* performance of the pellet gastric empty (PGE) test delivery system. This criticism has been addressed in the present proposal through the inclusion of a preliminary results section where we report the results of a preliminary study in normal human subjects, carried out in the past year, based on gastric motility monitoring and plasma level sampling. The proposed project would validate this delivery system using non-invasive salivary sampling and a second protocol evaluating the effect of meal components and NASA type meal on gastric emptying of the particles. The preliminary results indicate that in six out of six subjects the small particles empty ahead of the large particles as expected and while the subject number is small, the fact that the small particles emptied ahead of the large particles in all subjects validates the use of this test as a maximally discriminating test through the use of each subject being its own internal control (i.e., using the difference between large and small particle emptying administered at the same time to the same subject). Thus, this delivery system offers the maximum possibility of determining the effect of gravity on particle gastric emptying in a microgravity environment.

The primary objective of this proposal is to validate a novel non-invasive PGE test, utilizing salivary sampling developed in collaboration with NASA and The Upjohn Company, to assess particle size differentiation in gastric emptying and small intestinal absorption and intestinal and hepatic metabolism. The marker drugs employed in the PGE test, caffeine (CAF) and acetaminophen (APAP), serve as probes for gastric emptying and intestinal absorption as well as oxidative and conjugative metabolic changes. This noninvasive diagnostic, once validated in ground-based studies, will be utilized to measure the effects of microgravity on gastric emptying and intestinal absorption of drugs as well as intestinal and hepatic metabolism. Validation of the gastric emptying

rates with quantitative motility patterns will be performed with gastric catheterization, manometric monitoring, and simultaneous non-invasive sampling of saliva and urine following administration of PGE test. The results of the proposed project will be a validated noninvasive test system that can be used in a microgravity space station environment. The long term results of these studies will have direct implications for improved drug therapy and therapeutic protocols, as well as providing basic information on gastrointestinal function and adaptation in space. This evaluation of gastrointestinal function will provide basic information on drug and nutrient absorption and metabolism which in turn can be used to improve overall physical condition of crew members in a microgravity environment.

The tasks for 1996 include completion of the analysis and publication of the results of Study 1—validating the non-invasive salivary PGE test. The specific aims of this test were to:

1. Validate the use of a non-invasive method to assess differential fed and fasted state gastric motility based on the gastric emptying and absorption characteristics of 0.70 mm and 3.6 mm diameter enteric coated pellets containing marker compounds through direct measurement of the motility patterns from simultaneous gastric manometry measurements.

2. Confirm the similarity of gastric emptying and absorption when both marker drugs are administered in 0.70 mm enteric coated pellets.

3. Determine the pharmacokinetic parameters, including area under the concentration time curve (AUC), volume of distribution, time to reach maximum concentration (tmax), time to observe initial concentration (tini), absorption rate constant, elimination rate constant, elimination half-life, and peak concentration (Cmax) in relation to the differential emptying and absorption patterns of each of the marker drugs from plasma and saliva samples.

Preliminary results indicate that the small particles emptied ahead of the large particles in every subject. In addition, the emptying rates were correlated with gastric contractions indicating return to the fasted state for the larger particles. Thus, the emptying of the large particles serves as a marker of meal emptying time while the small particles emptying more rapidly than the large particles indicates that the hydrodynamic discrimination expected is achieved in these ground-based studies. During the coming year, Study 2—influence of nutrient type on gastric emptying, absorption and metabolism as determined by the simultaneous measures of gastric motility via the non-invasive PGE test and gastric catheterization—will be initiated.

A. Hypotheses:
1. Gastric emptying can be significantly influenced by nutrient and meal composition. The non-invasive PGE test can be utilized to discriminate the effect of specific nutrients on gastric emptying and drug absorption.
2. Nutrient type will influence the metabolism of caffeine and APAP serving as markers for specific hepatic oxidative and conjugative metabolic pathways based on urine metabolite profile measurements.

Specific Aims:
1. To assess the influence of meal composition on gastric emptying and absorption from the isocaloric administration of individual nutrients: glucose, fat, or amino acids versus a standard breakfast.
2. To compare the pharmacokinetic parameters, including area under the concentration time curve, volume of distribution, time to reach maximum concentration rate constant, elimination half-life, peak concentration (Cmax), and relative biavailabilities for acetaminophen and caffeine in relation to meal composition.
3. To validate the utility of non-invasive saliva sampling as a means to assess gastric emptying and absorption.
4. To evaluate the influence of nutrient composition on the oxidative and conjugative metabolic pathways specific for acetaminophen and caffeine from urine profile analysis.

This project will evaluate a new, non-invasive test to assess gastric emptying, the PGE test. This test could be used to assess gastric emptying here on earth as well as in outer space. Alterations in gastric empty occur in many disease states, including diabetes mellitus, AIDS, critical illnesses, viral infections, and gastroesophageal

reflux disease. Current methods to assess gastric emptying include manometry and or scintigraphy. These methods are invasive, cumbersome, associated with risks, and costly. The new PGE test would allow for easy, serial, non-invasive assessment of gastric emptying in man.

Adaptive Visual-Vestibular Mechanisms and Gravity

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-16-17-13
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $80,689
Students Funded Under Research: 1

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 be sensory conflict between the visual and vestibular signals. It is hypothesized that, after adaptation to a tilted visual environment, the vestibular system still monitors Earth-vertical via the otolith receptors; however, this spatial vertical is no longer aligned with the gravitational force, but rather shifted in the direction of tilt of the visual world. The proposed project will provide information about integration and fusion of multisensory inputs and adaptive changes in gravity-sensitive central mechanisms. Such information about adaptive changes in the central vestibular system during conflicting, non-complementary visual-vestibular interactions are important for a better understanding of similar adaptive changes that occur in microgravity during space flight.

Since the beginning of the funded period, the primate laboratory is fully functional and equipped with a unique three dimensional motion delivery system that can provide any combination of linear and angular motion stimuli

in three dimensions. Experimental animals have been implanted for experiments and trained for three dimensional eye movement recordings. Experiments associated directly with the specific aims of the grant are currently in progress and several interesting results are being obtained during preliminary analyses of the data (see below). In addition, on-going work on topics directly relevant to NASA interests about the role of gravity-sensitive responses on the vestibulo-ocular reflex have been completed and published in peer-reviewed journals (see Publication list).

A main goal of the funded project is to study the ability of the vestibular system (semicircular canals and otoliths) to adapt to altered sensory conditions as registered by retinal slip associated with non-complementary visual stimulation. In the laboratory this is easily achieved by rotating the animals about an axis (i.e., yaw), whereas an optokinetic stimulus is being presented about a different axis (i.e., pitch). When the axis of rotation is maintained Earth-vertical throughout the motion, only semicircular canal receptors are dynamically stimulated. When the axis of rotation is Earth-horizontal, both semicircular canals and otolith receptors are stimulated. Following repeated exposure to these conditions, the vestibular system "learns" to generate a pitch (vertical) eye movement during subsequent yaw rotations in complete darkness.

Preliminary analyses have shown several interesting results: (a) When only the semicircular canal system is asked to adapt, the adaptive properties are uniform for all directions of motion and optic flow stimulation; (b) When also the otolith system is activated, the adaptive properties of the vestibulo-ocular system change; certain combinations of vestibular/visual directions are easier coupled than others; (c) Whenever the otolith system is dynamically activated during adaptation to the non-complementary vestibular/visual conditions, the extent of speed of adaptation is augmented, particularly during low frequency motion; (d) The increased contribution of the otolith system to vestibular/visual adaptive properties is even more conspicuous in the fact that it is able to adaptively "learn" to generate an orthogonal eye movement at a different frequency from that of the associated head and body motion. When, for example, the accompanying visual stimulation occurs at a frequency that is double from that of head rotation, the orthogonal component that the system learns to generate during subsequently motion in complete darkness is at this double frequency.

These preliminary results are very important for our understanding of visual/vestibular interactions during normal and non-complementary sensory conditions. Continuing experiments and more detailed analyses will further increase our understanding of these properties. In normal behavior, as well as in the extraordinary challenges of altered gravity, spatial orientation and motor coordination are intriguing tasks due to their complexity and the need for integration of movement information from several sensory modalities including the vestibular, visual, and somatosensory systems. Even for a single system, as for example the vestibular system, the otolith and semicircular canals provide distinct information which must be centrally fused, integrated, and re-interpreted. Our efforts so far have provided interesting experimental results and theoretical insights into the process of extracting motion information from a combination of otolith and semicircular canal information.

The research funded by this NASA grant aims to understand basic mechanisms underlying the normal organization and coordination of otolith, semicircular canal, and visual signals. By comparing the normal with the adapted visual/vestibular mechanisms underlying eye movement control and spatial orientation, these studies aim at improving our understanding of such multisensory integration questions in normal and disease states not only on Earth but also in space where altered gravity/visual interactions provide a demanding challenge on our cognitive and motor functions. Results of these studies will be important in understanding the process of sensory adaptation to altered visual/vestibular conditions experienced during space travel and upon return to the Earth's gravitational environment.

FY96 Publications, Presentations, and Other Accomplishments:

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Neural Plasticity: Data and Computational Structures [Human Brain Project]

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Funding:

Project Identification:
Solicitation:
Initial Funding Date: 9/95
Expiration: 8/96
FY 1996 Funding: $1,136,409
Students Funded Under Research: 33
Joint Agency Participation: NIMH, NIDA

Task Description:

This 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. We are integrating research at seven different laboratories for neuroscience; 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.

A key idea of the work is that we are federating databases of the following four kinds:

• Repository of Empirical Data (RED): protocol-wrapped data from experiments.

• Summary Data Base (SDB): basic assertions, summaries, hypotheses, tables, figures, etc., for some domain of knowledge.

• Article Repository (AR): narratives - journal articles, chapters, hypertext documents - to provide threads through the databases.

• Model Repository (MR): links computational models to empirical and summary data bases.

Databases developed at USC will provide both (i) seeds for the development of databases whose data will come from many sites at USC and elsewhere; and (ii) exemplars to develop databases elsewhere which can be linked into a federation of which the USC databases will form just one part. Our work is designed to encourage the sharing of the development and application of informatics tools across the U.S. and international neuroscience community.

In FY 96, we restructured our Project into four research thrusts:
1) Brain Models on the Web
2) Data Management and Mining
3) Time Series Databases
4) Visualization and Atlas-Based Data

What follows is a brief statement of the work in each thrust area, and a listing of the key personnel involved. Further information, as well as access to sample tools, databases, and simulations, may be found by following the links from our home page at URL http://www-hbp.usc.edu/.

1) Brain Models on the Web

This Thrust focuses on modeling, and the development of Brain Models on the Web as a Model Repository. Michael Arbib (PI and Director of the USCBP; Professor of Computer Science, Neurobiology, and Biomedical Engineering) directs the development of our database Brain Models on the Web and of USC's Neural Simulation Language NSL, as well as systems level modeling of a variety of brain regions and behaviors. Theodore Berger (co-PI; Professor of Biomedical Engineering and Neurobiology) conducts electrophysiological and neuropharmacological research on hippocampal slices and related synaptic modeling. Amanda Alexander (Systems Programmer) is the architect of NSLJ, a new version of NSL written in Java to maximize Web access to brain models. Jim-Shih Liaw (Research Assistant Professor of Biomedical Engineering) works on synaptic modeling, and is developing EONS, a library of Elementary Objects of the Nervous System for use with NSL.

2) Data Management and Mining

This Thrust develops mechanisms for discovery and data mining for all types of databases, but especially for Web-accessible Article Repositories, and examines how the data so retrieved may be annotated and organized into Summary Databases. Dennis McLeod (co-PI, Professor of Computer Science) directs work on database federation, conceptual modeling, and dynamic ontologies which extend the notion of an adaptive thesaurus. Thomas McNeill (co-PI, Professor of Gerontology) directs work on intelligent searching, and works with Arbib on the definition of summary databases.

3) Time Series Databases

This Thrust focuses on Repositories of Experimental Data gathered through neurophysiological experiments. The key concept is that time-series data be associated with a protocol which defines the experimental

manipulations and conditions under which the data were obtained - such protocols can also be used to design interfaces for computational modeling. Our approach to time-series data (both neurophysiological and behavioral) is to define a core database structure that is readily expandable to meet the needs of diverse laboratories. The generality of this structure is tested by its application both to a database of recordings from hippocampal slices in the Berger laboratory, and the study of cerebellar correlates of classical conditioning in the laboratory of Richard Thompson (co-PI, Professor of Psychology and Neurobiology). The Core Database Structure was designed by Jeff Grethe (Doctoral Candidate in Neurobiology) and implemented with the aid of Jonas Mureika (Systems Programmer).

4) Visualization and Atlas-Based Data

This Thrust focuses on Repositories of Experimental Data in which data need to be visualized in relation to anatomical structure. Larry Swanson (co-PI, Professor and Director of Neurobiology) has developed the atlas of the rat brain which provides the basic templates for our approach to atlas-based data. Swanson works with Shahram Ghandeharizadeh (co-PI, Associate Professor of Computer Science) to develop NeuArt, a neuroanatomical viewer which enables flexible viewing of a vast array of data as overlays on templates from the Swanson Atlas. Michel Baudry (co-PI, Professor of Neurobiology) and Georges Tocco (Research Assistant Professor of Neurobiology) are using this framework to develop a neurochemistry database, warping autoradiographic slices into 3-D volumes reconstructed from the 2-D templates of the Swanson atlas, and providing data on receptor density to constrain modeling conducted by the Berger group.

In administering the Project, Dr. Arbib is assisted by Paulina Tagle (Administrative Coordinator) and Luigi Manna (Computer Laboratory Manager).

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. Moreover, our research on atlas-based data on the brain has useful parallels to work on Geographical Information Systems.

Adrenoreceptor Hypersensitivity in Models of Weightlessness

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Project Identification: 199-08-17-61
Initial Funding Date: 2/96
FY 1996 Funding: $120,000

Solicitation:
Expiration: 2/97
Students Funded Under Research: 4

Task Description:

Our central hypothesis is that alterations in autonomic function occur because of exposure to microgravity, and that these alterations are likely responsible for many of the physiological responses to weightlessness. Our overall aim remains to understand the alterations in the autonomic nervous system observed in models of weightlessness. Two models are being studied: bedrest deconditioning, and patients with orthostatic intolerance. In the current funding year we have established state of the art techniques in our laboratory to determine the effects of bedrest on sympathetic function. Our preliminary studies also provide evidence of hyper-responsiveness of adrenergic agonists in patients with orthostatic intolerance. These patients have a clinical picture similar to that observed in astronauts upon return to 1-G.

In the past year we have expanded our observations in clinical conditions characterized by orthostatic intolerance (OI). The purpose of these studies was to determine potential pathophysiological mechanisms of OI. In particular, we wished to determine the contribution of plasma volume, autonomic, and renin mechanisms in OI. We find an exaggerated postural fall in plasma volume in patients with idiopathic OI. We also find an increased cardiac sensitivity to 1 adrenoceptor agonists and increased vascular sensitivity to 1 adrenoreceptor agonists in these patients. Furthermore, they have decreased sensitivity to the norepinephrine-releasing effect of tyramine. Finally, we have found that patients with diabetes mellitus can also present clinically with OI which is very similar to that observed on astronauts in the immediate post-flight period. It is hoped that better understanding of the OI associated with weightlessness will ultimately lead to better treatment for these common and important clinical conditions such as idiopathic OI and diabetes mellitus.

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. Our recent studies have also characterized a similar orthostatic intolerance to that of astronauts also occurs in some patients with diabetes mellitus. It is hoped that understanding the mechanisms of orthostatic intolerance will lead to improved treatment of this important disease.

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Otolith and Vertical Canal Contributions to Dynamic Postural Control

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Solicitation: 95-OLMSA-01
Initial Funding Date: 9/93
Expiration: 9/96
FY 1996 Funding: $174,130
Students Funded Under Research: 1

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. William H. 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 also 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 is 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 may not be possible until this problem is solved.

Results to date strongly suggest 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.

During years 2 and 3, we completed a pilot project designed to determine the frequency-dependent dynamics of visual-vestibular (VVOR) interactions. Results support the hypothesis that VVOR interactions are non-linear in normal subjects, but become linear under some conditions in abnormal subjects. For example, when VOR and OKN functions are normal, a non-linear "saturation constant" (e.g. "unity gain") appears to govern VVOR interactions. However, when a critical gain threshold is reached in either the VOR or OKN system, interactions appear to become algebraic, but inadequate for compensation, until function is lost. Phase relationships are more complicated and will require further study.

Normal subjects are being recalled from a previous, large N (>200), cross-sectional (as a function of age) study of horizontal canal vestibuloocular (HCVOR), optokinetic (OKN) and dynamic postural (DP) control function tests. To date, 52 of these normal subjects have been recalled and have undergone the rebaseline protocol. Most of these subjects are now in the next age decade. The numbers are still too small in each group (decade) to assess test-retest variability. However, results to date strongly support an age-related decline in vestibular function detectable by our methods at about age 40.

All records from our normal population studies (N>200) underwent retrospective analysis. Results were reported in detail in the 1995 progress report for NAGW-3799. These results will be included in the final report and peer reviewed publication(s) (to be submitted) on normal subject VOR and VS responses to complex stimuli.

Patients scheduled for labyrinthectomy, vestibular nerve section, and/or acoustic neuroma (schwannoma) resection underwent preoperative baseline testing. Results of this experiment, which is an expansion of work in progress, will contribute to a better understanding of the mechanisms underlying compensation for abnormal vestibular function. Results from this portion of the project will provide data for ground-based data for comparison with returning astronaut data.

In 1996, we reported on the minimal vestibular function required for compensation after complete unilateral or bilateral loss of vestibular function. We found that compensation of vestibulospinal function does not necessarily accompany VOR or VVOR compensation. We surmise that ascending (VVOR) and descending (vestibulospinal) routes of compensation may involve different adaptive processes.

Technical Progress

Hydraulic Pitch Axis Rotation Device. An existing hydraulic actuator is being modified for pitch and roll stimuli.

Human-rated Centrifuge. The human rated centrifuge has been completed and initial data collection has begun.

Video eye movement recording and analysis techniques. The binocular Clark VOG 3-D video eye movement recording and analysis system has been purchased and has undergone check-out and calibration procedures. A side-by-side comparison between conventional electro-oculography and the new infrared video system is complete and data analysis underway.

Modified dynamic posturography device. During the coming year, modification of the computerized dynamic posturography devices will be completed.

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 very costly. Vestibular disorders are a common cause for falls in the elderly. Spatial disorientation results in significant loss of life and equipment in military aviation each year. Results of NASA sponsored vestibular research projects have produced the only large N normal data bases and have contributed significantly to our understanding to vestibular adaptive and compensation processes. Results of space flight experiments have permitted, for the first time, the determination of recovery dynamics of the human vestibulospinal system following exposure to novel inertial environments.

NASA sponsored research has brought us much closer to diagnostic methods and treatment of debilitating problems, such as mal de debarquément following cruises (which are very disabling for the elderly), Meniere's disease, recovery from acute vestibular insults, avoidance of ototoxicity, and common motion sickness.

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 gravitational 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 otolith 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 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 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. Equitest® CDP is the first, clinical postural control assessment method to receive a CPT code.

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 EquiTest® postural control assessment system is the only method of its 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 has become a part of the routine medical assessment of astronauts returning from long duration space flights. In addition to diagnostic and assessment uses, we anticipate that future developments will assist clinicians in the selection and monitoring of patients undergoing treatment and rehabilitation regimens and may detect persons at risk for falls in the aging.

FY96 Publications, Presentations, and Other Accomplishments:

Black, F.O. (co-chair) Vestibular dysfunction: Lessons and legacies from space. AAO-HNS / NASA, Washington, D.C. (1996).

Black, F.O. (invited exhibit/organizer) NASA Life and Biomedical Sciences and Applications Division/NIDCD Communications Awareness Day Exhibit for Congress, Washington, D.C. (October, 1995).

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Neuronal Vulnerability and Informatics in Human Disease [Human Brain Project]

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Funding:

Project Identification: n/a
Solicitation:
Initial Funding Date: 6/96
Expiration: 3/97
FY 1996 Funding: $50,000
Students Funded Under Research: 6
Joint Agency Participation: NIH and Human Brain Project

Task Description:

To facilitate the implementation of the software development, an Administration Core will develop and coordinate the computer technologies among the different research projects. The Core will work closely with all five projects in developing not only the precise kinds of equipment that each project needs to complete its scientific goals, but to produce a common foundation of technology to be used by the projects in order to produce a seamless integration of communication and data among the Projects. As new enabling and emerging technologies in computers, networking, and communications occur in the computer sciences and in the software developments of this P20, the Core will have the responsibility to evaluate their potential application to the science being conducted by the projects. The Administrative Core will have four specific aims: 1) the Core will be responsible for connecting all computer platforms in each of the four projects of this Consortium together. Both TCP/IP and DDP protocols will be fully supported such that all clients, servers, and applications that the Consortium will develop can be executed or accessed from any computer in the Consortium and to other investigators outside the consortium as software development refinements are found acceptable; 2) the Core will work with other projects in developing computer technologies. Specifically, these are the NeuroZoom systems of the Morrison Project, and the NeuroBase, NeuroAtlas, and NeuroNet systems of the Bloom/Young Project. These technologies will then be distributed to all other projects of this Consortium; 3) the Core will work with the other projects in incorporating their data into electronic form suitable for NeuroZoom, NeuroBase, NeuroAtlas, and NeuroNet. Specifically, this includes the Jones Project for the electronic digitization of the Jones and Berman Macaca mulatta brains and the Bloom/Young Project for the production of the electronic Mannen cat brain atlas; and 4) the Core will work closely with other collaborating P20 and RO-1 applicant groups responding to the Human Brain Project Program Announcement to create the necessary data filters, communication interfaces, and data structures with the overall goal of inter-laboratory data sharing and communications. Thus, the Administrative Core will be specifically responsible for implementing (distributing, installing, and training) the data acquisition, data analysis, database, and communication software as they are developed and for assuring that the collaborating projects can use them appropriately.

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 2 1/2 years of funded operations, we have concentrated on two aims: 1) software development and 2) system/hardware confirmations. At this point the main software, NeuroZoom, has been written and is being seeded to users.

Completing the NeuroZoom software - NeuroZoom is a microscopy data acquisition and analysis software program for the Apple Macintosh computer system, designed to support tissue mapping from any kind of microscope using traditional topographical mapping techniques and with statistical stereological probes. NeuroZoom was designed from the ground up to be first and foremost a mapping program. By controlling the movement of the XYZ axes on the microscope and displaying live video images in a computer window with data aligned on it, NeuroZoom is capable of extracting 3-D data from any visualized object. Typical uses of this are creating distribution maps of molecular markers on sections of biological structures.

In Year 4, stereological probes have been added to NeuroZoom to assist in the unbiased quantification of cellular counts, nuclear volumes, cellular volumes, and surface areas. The database engine developed in Year 3 has been implemented into the architecture of NeuroZoom so that collected data may be published outside of the local computer that NeuroZoom is running on to other collaborators via the Internet. In addition, a rudimentary framework for a neurocircuitry database is being prototyped with this database engine that focuses on cellular morphology of brains and the interconnections among them. Using either NeuroZoom itself, or another software application developed specifically as a client application to this database, Internet users can log into collaborative databases to view collected, synthetic, and reduced data. Models include raw data based on topographical maps, stereological estimates of counts, surface areas, and volumes, and of connectional information that arise from both conclusions drawn from experiments conducted with NeuroZoom, as well as from encyclopedic investigations of knowledge in the database or from the literature. All software development was done by Dr. Warren Young (Scripps), also directing all programming efforts, and the programmers, Ms. Soraya Gonzalez (Scripps) and Mr. Harry Stern (Mount Sinai).

Using NeuroZoom to create rudimentary brain atlases - To support the visualization of neurocircuitry data with atlases, brains have been processed and captured digitally. The basic steps to making an atlas have been outlined as using MRI to represent the brain before any deformation. If MRI of an animal is not possible, microtome sections from the frozen block face are captured at high resolution before knife sectioning. The block faces suffer from some distortion as part of the freezing and/or embedding process, but are close enough to serve as fiducial registration controls.

Data Collection - As experimental data are collected using NeuroZoom, local areas of data represented by topographical mapping and morphometry, unbiased stereological estimates of cell counts, and boundaries begin to grow. Since atlas templates are in the database, a user can associate his experimental data to locations defined stereotaxically in the atlas templates. A NeuroZoom window with the appropriate section of the atlas is opened and displayed. The experimental data are acquired from sections that are aligned in the appropriate plane of section of the atlas template. The user then judges where in the atlas the experimental data should be associated. Selecting the data and dragging it to the templates makes the spatial association.

Data that are collected using NeuroZoom stereological protocols are more efficient than broadly mapping all structures using traditional mapping and morphometric techniques. Calculations of variance and coefficient of error (CE) are part of every analysis and are useful for optimizing studies. Each data report includes information regarding the configuration of the microscope, objectives, stage, counting frame if relevant, and other variables in use during data acquisition, so that the quantitative data obtained can be interpreted in context long after the session is over. Like the mapping data, these stereological data may also be associated with an atlas template.

The data may also be stored without template information. What begins to grow in this particular instance of the database is a quantitative representation of profile counts of different regions of the brain. Because the data

were collected in an unbiased stereological manner, it is less immune to histological variability, and reflects more accurately a usable numeric database. However, because NeuroZoom always has the capability of mapping standard topographical data while it is collecting stereological data, both the unbiased numeric counts and structural information can be stored together.

On the most macro of all levels, one can use the atlas templates derived from whole brain sections and retrievable with NeuroZoom to see stereotaxically placed data, including stereological data. However, one can also build a stereological database with a minimal amount of structural detail.

If stereological data are gathered with the boundary information detailed by the topographical tools in NeuroZoom, these can serve themselves as a regional atlas. The Lateral Geniculate Nucleus can be stored in the database with the cellular profile numbers estimated with stereological tools, but localized in a mapping system with the layers outlined with the topographical tools. In this way, both whole brain normative atlases, as well as regional atlases of specific structures can be stored in the database and made accessible to others over the Internet.

Significance - NeuroZoom has been installed at the Department of Neuropharmacology, The Scripps Research Institute; Fishberg Center for Neurobiology; Mt. Sinai School of Medicine; Department of Neurosciences, University of California, San Diego; Washington University, St. Louis; and Rush Presbyterian Hospital in Chicago. We had a successful showing at the 1996 Society for Neurosciences meeting in Washington, DC, and have created a specialized Web site to support the distribution and use of NeuroZoom. Given the interest expressed at our abstract poster displays at the 1996 Society for Neuroscience meetings, we expect that there will be many scientists downloading and using the software. Since NeuroZoom was designed for overall general use in topography and stereology, scientists from outside of basic neurosciences will also be encouraged to use the software. We have compared NeuroZoom to 4 other commercial packages during the 1996 annual meeting of the Society for Neuroscience, and have found that the feature set in NeuroZoom is far more complete and advanced that all others. The stereology is coupled to topographical mapping, which only one other commercial software, BioQuant, can claim as a feature. However, Bioquant, at $18000, provides only one stereological tool, the optical dissector, for estimating cell counts, while we provide tools for global and regional volumes and surface areas. Furthermore, we are the only software package that is coupled to a networked database that can act as a local or globally accessed system.

The work on NeuroZoom as an acquisition tool using stereological principles, and the development of a database with stereological data has also led to the submission of a STTR grant proposal by Floyd Bloom. A stereologically-derived database of key mouse brain structures in the normal brain will be developed and compared against transgenic mouse models of spinal neurodegeneration. The intent is to provide a normative model for pathology in genetically-manipulated mice using unbiased stereological data.

We feel that the contributions from use of NeuroZoom will be immense, providing easy to use stereology tools to collect data in an unbiased manner. The Journal of Comparative Neurology recognizes the importance of collecting data in an unbiased manner, and is requesting that all papers submitted to their journal use these techniques when collecting quantitative data. These data may then be compared in similar unbiased manner to data from other collaborators, contributing to a growing database of quantitative neuroanatomic knowledge.

Plans - Year 5 will see the release of NeuroZoom into the scientific community. Documentation is being completed, existing software bugs are being eliminated, and a support system is being put into place as part of the specific aims of the Administrative Core. Currently, support for NeuroZoom is very important. This is not a small, simple application. NeuroZoom controls electronic equipment, such as motorized microscope stage systems. The microscope needs to be properly configured and connected to the computer running NeuroZoom. Furthermore, if the stereological protocols are to be used, a good strong working knowledge of stereological principles are required in order to produce the best results. To this end, Dr. Esther Ninchinsky and Dr. Patrick Hof are writing up documentation on the principles of stereology, and how to use stereology within the context of NeuroZoom. Both basic principles and task oriented chapters are being written. These and the basic manuals

to support other features of NeuroZoom are in a portable document format that any computer system can open. Documents are retrievable from the NeuroZoom Web site, and are also viewable directly from the Web browser.

The database framework will also be expanded in Year 4. Prototyping will continue using the database engine developed in Year 3. However, there will be support for users from the Web via HTML (Hypertext Markup Language) queries to server based gateways known as CGIs (Common Gateway Interfaces) to the database engine. Since HTML and the generation of forms as presentation of database content to the user is limited, there will also be the development of JAVA applets that execute both within the Web Browser, and as standalone applications on the remote client user side. JAVA is a new, modern, object oriented language, loosely based on the syntax provided by C and C++, but with the advantages offered by Smalltalk and other dynamic languages. JAVA is not only a modern language designed from the ground up, and with a tremendous amount of vendor support and momentum, but also cross-platform, meaning that it will operate without incurring a lot of changes in the software on other computer systems. With JAVA, the client end of the database system can be extended to Windows 95 and Windows NT users initially, and later extended to UNIX users. Once the JAVA client software is in place, the database engine will be replaced with a JAVA interface surrounding a ODBC (Open Database Connectivity) compliant SQL (Structured Query Language) relational database. The database will be used as a repository only, storing and retrieving persistent forms of the objects to the JAVA object handlers. The database will remain object oriented such that inheritance, persistency, polymorphism, and other attributes of modern object oriented languages are fully exploited in the database.

To support the release of NeuroZoom, a short beta period is planned for the first half of 1997. There will be no restrictions placed on who can download the software. A bug reporting system is in place for reports sent in by users. Questions will be answered as soon as possible by e-mail. Following a bug free period will be the first full release (we anticipate summer 1997). This software will be serialized so that use of NeuroZoom can be tracked, and the users better supported in terms of software patches and updates.

1. Does this research seek to understand a disease or malady that affects humans on Earth and/or in space? *Yes —the common elements that can lead to loss of brain functions by death of nerve cells (neurons).*

2. Does this research seek to develop new therapeutics or protocols for alleviating symptoms of a disease or malady on Earth? *Not in any direct way.*

3. Will this research yield a new understanding of basic biological processes? *Yes—this research can define how individual subjects may become vulnerable or resistant to environmental challenges that can accelerate death of neurons.*

4. What relationship does this task posit between processes on Earth and in space? *None specifically.*

5. What impact could the results of this research have on the common man? *Better understanding of the brain's capabilities in health and disease.*

6. What benefits are foreseen by the development of this new technology? *New ways to link chemistry, anatomy, physiology, and treatments with specific cellular components of the brain.*

FY96 Publications, Presentations, and Other Accomplishments:

Akbarian, S.A., Huntsman, M.M., Kim, J.J., Tafazzoli, A., Potkin, S.G., Bunney, Jr., W.E., and Jones, E.G. GABAA receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. Cerebral Cortex, 5, 550-560 (1995).

Akbarian, S.A., Smith, M.A., and Jones, E.G. Editing for an AMPA receptor subunit RNA in prefrontal cortex and striatum in Alzheimer's disease, Huntington's disease and schizophrenia. Brain Res., 699, 297-304 (1995).

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Hof, P.R. and Morrison, J.H. Neurofilament protein defines regional neurons of the human anterior cingulate cortex. J. Comp. Neurol., 355, 27-37 (1996).

Hof, P.R., Mufson, E.J., and Morrison, J.H. Human orbitofrontal cortex: Cytoarchitecture and quantitative immunohistochemical parcellation. J. Comp. Neurol., 359, 48-68 (1995).

Hof, P.R., Ungerleider, L.G., Webster, M.J., Gattass, R., Adams, M.M., Sailstad, C.A., and Morrison, J.H. Neurofilament protein is differentially distributed in subpopulations of corticocortical projection neurons in the macaque monkey visual pathways. J. Comp. Neurol., 376, 112-127 (1996).

Longson, D., Stubbs, C.M. and Jones, E.G. Localization of CAMIIKinase-a, GAD, Glu-R2 and GABAA receptor subunit mRNAs in human entorhinal cortex. J. Comp. Neurol., (in press).

Macchi, G. and Jones, E.G. Towards an agreement on the nomenclature of the human motor thalamus. J. Neurosurg, (in press).

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Nimchinsky, E.A., Vogt, B.A., Morrison, J.H. and Hof, P.R. Spindle patterns of cortical organization in the macaque monkey visual system: A quantitative immunohistochemical analysis. J. Comp. Neurol., 352, 161-186 (1995).

The Role of Vestibular Information in Adaptive Modification of Eye, Head, and Hand Coordination

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Funding:

Project Identification: 199-16-11-55
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $ 109,000
Students Funded Under Research:

Responsible NASA Center: JSC

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 a fixed target displayed on a featured background. We then compared subject's pointing accuracy following whole-body rotation in the light versus the dark. We presented the results of the second study at the Society of 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 entitled "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.

The results of our third study provide empirical evidence that adaptive alteration to VOR function produces corresponding alterations in vestibular contingent pointing responses. Subjects were exposed to a 30 minute

adaptive stimulus to modify VOR function. When comparing pre and post-adaptation responses, subjects suffered significant decrements in performance in locating target position after the adaptive stimulus. This study also provides evidence that VOR gain can be adaptively reduced by 20% in a 30 minute period. A manuscript is currently being prepared for submission. An abstract of this work is being prepared for submission to the 1997 Society for Neuroscience Annual Meeting in New Orleans.

Through these investigations, we have answered these questions: Will differences in the visual context of target display affect the subjects pointing accuracy? Can vestibular information alone be used to spatially code manual pointing responses? What is the minimum exposure time to minifying lenses needed in order to reduce the vestibulo-ocular reflex (VOR) gain by 20%? Can adaptive alterations in eye-head coordination produce commensurate alterations in the ability to manually locate target positions?

We are also beginning a new experiment to further elucidate the role of vestibular signals in the spatial coding of saccadic eye and manual pointing movements. The protocol is similar to those used previously in our laboratory and involves pointing to a remembered target location after rotation in the dark, without the aid of visual cues. Without vision, vestibular cues are the primary source of information to program coordinated eye, head and hand movements. The unique aspect of this study is the incorporation of labyrinthine deficient patients into our sample. We expect that without a vestibular signal, this sample will be unable to perform the task in darkness.

The results of these experiments serve as the foundation for our on-going investigation aimed at determining how the oculo-motor and manual systems share information, and how this information is susceptible to common adaptive distortion following exposure to conflicting visual-vestibular stimuli.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Barry, S.R., Bloomberg, J.J., and Huebner, W.P. The effect of visual context on manual localization of remembered targets. Neuroreport, 7, (1996).

Huebner ,W.P. and Bloomberg, J.J. A system for the accurate measurement of pointing responses. J. Neurosci. Methods, 64, 233-236 (1996).

Biochemical Adaptations of Anti-Gravity Muscle Fibers to Disuse Atrophy

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Funding:

Project Identification: 199-26-17-05
Initial Funding Date: 3/94
FY 1996 Funding: $87,000

Solicitation:
Expiration: 2/97
Students Funded Under Research: 10

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 do so. However, we have tested two other genes not initially proposed. First, based upon findings in our laboratory from a NIH grant, we tested a 3'-UTR region of the cytochrome c mRNA whose RNA-protein interaction had been shown to be: a) decreased when contractile activity of low oxidative muscle was increased, and b) low in the soleus muscle compared to a low oxidative muscle. 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.

We examined the association of IGF-I expression with atrophy in skeletal muscle. Dr. Vernikos (*BioEssays* 18:1029-1037, 1996) published a paper in which literature citations lead to the hypothesis that IGF-I mRNA would be decreased in muscles of non-weight bearing hindlimbs. Surprisingly, we found that the relative abundance of endogenous IGF-I mRNA in the gastrocnemius muscle was unaltered by 14 days of non-weight bearing in the mouse. This complements our previous report that indicates that skeletal muscle IGF-I mRNA expression in fast-twitch muscle was unchanged in the atrophic muscles of old rats. Our interpretation is that whereas increased IGF-I mRNA expression may be involved in skeletal muscle hypertrophy, it does not seem to be causal for non-weight bearing atrophy of skeletal muscle. Concurrent with this study we performed a study to determine whether over-expression of IGF-I would abate or prevent the non-weight bearing muscle atrophy. Male transgenic and non-transgenic mice from the parent strain (FVB) were divided into four groups (n = 10/group): 1) transgenic, weight bearing (IGF-I/WB); 2) transgenic, hindlimb non-weight bearing (IGF I/NWB); 3) non-transgenic, weight-bearing (FVB/WB); and 4) non-transgenic, hindlimb non-weight bearing (FVB/NWB). Non-weight bearing groups were hindlimb non-weight bearing for 14 days. Body mass was reduced ($P<0.05$) following non-weight bearing in both transgenic IGF I mice (-9%) and FVB mice (-13%). High level expression of IGF-I mRNA was confirmed in the gastrocnemius and tibialis anterior muscles of the transgenic mice. Nevertheless, the mass of the gastrocnemius and tibialis anterior muscles was reduced ($P<0.05$) in both FVB/NWB and IGF I/NWB groups compared to FVB/WB and IGF-I/WB, respectively, and the percent atrophy

in mass of these muscles did not differ between FVB and IGF-I mice. Therefore, a high level local expression of IGF-I mRNA in mouse skeletal muscle does not prevent non-weight bearing induced atrophy of fast-twitch muscle. Because the transgene was not expressed in the soleus muscle of transgenic mice over expressing IGF-I, we were unable to test the effect of its over expression in slow-twitch muscle.

The size of skeletal muscle determines the ability to perform manual work. Skeletal muscle loses one-half of its mass by the age of 80 years in humans. In many cases, this results in humans losing their ability to care for themselves, i.e., they do not have the ability to accomplish the activities of daily living. Humans lose 10% of their muscle mass from ages 25 to 50 years and lose an additional 40% of their muscle mass from ages 50 to 80 years. In the model of hindlimb non-weight bearing, the amount of muscle mass lost in years in humans is condensed to weeks. After one week of hindlimb non-weight bearing, mice have losses of 10–20% in skeletal muscle mass. Space flight also offers a laboratory to accelerate the loss of muscle mass and to determine why humans lose muscle mass with aging. Loss of skeletal muscle also occurs in many illnesses, such as AIDS, diabetes, obesity, congestive heart failure, etc. NASA studies into muscle atrophy can be considered as nearly the sole source for this research problem as NIH supports little research into muscle atrophy.

This research is also attempting to determine whether the upregulation of IGF-I expression could be a countermeasure to muscle atrophy produced by non-weight bearing of muscle. If successful, IGF-I would be a new therapeutic for preventing muscle atrophy on Earth. If methods of prevention of skeletal muscle atrophy can be found for humans, the quality of life would be enhanced by delaying the entry of people into nursing homes because of physical frailty and by speeding the rehabilitation of skeletal muscle during many clinical diseases. An additional benefit is the reduction of health care costs, a problem which will increase as more Americans reach the age of frailty.

FY96 Publications, Presentations, and Other Accomplishments:

Booth, F.W. and Baldwin, K.M. "Muscle plasticity: Energy demand/supply processes" in "Handbook of Physiology: Section 12: Integration of Motor, Circulatory, Respiratory, and Metabolic Control during Exercise." Edited by: Rowell, L.B. and Shepherd, J.T. Oxford University Press/New York, pp 1075-1123, 1996.

New Statistical Methods for Immunoassay Data Analyses

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-70-17-19
Initial Funding Date: 6/95
FY 1996 Funding: $138,990

Solicitation: not applicable
Expiration: 5/97
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; and (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; and (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 for FY96 are:

(1) the development of a new approach to modeling interassay variation in routine immunoassay analyses;

(2) a new five-step paradigm based on Bayesian statistical theory for immunoassay quality control, calibration and measurement (QCCM);

(3) a comparison of the new QCCM paradigm with Levy-Jennings 2s and Westgard multirule methods for quality;

(4) a method for validating quality control setup criteria;

(5) further improvement in our Monte Carlo Markov chain algorithm for computing the QCCM paradigm;

(6) successful analysis of parathyroid hormone data; and

(7) successful analysis of melatonin immunoassay data.

The research represents the development of new statistical techniques to analyze immunoassay data. These methods should have broad applications in clinical and laboratory medicine because immunoassays are the most widely used procedure for measuring the concentrations of analytes in biological specimens. The primary benefits of these new methods will be on Earth; however, they may be used to analyze immunoassay based measurements of biological specimens collected during space missions.

The new technologic benefits from the research include: 1) a new method for determining the accuracy of any immunoassay measurement; 2) a new method for setting standards for immunoassay quality control; 3) a new approach for accurately defining the smallest concentration an immunoassay can measure; 4) new criteria for optimal design of immunoassays; and 5) PC-based software to apply the new methods. The health-related benefits of the new methods apply to any immunoassay based procedure. They are the establishment of more statistically rigorous standards for defining positive test results for disease screening and diagnostic medical tests, and for measuring reliably any analyte concentration with an immunoassay. Because the measurement of analytes with calibrated methods (spectroscopy, chromatography, and quantitative PCR) is an important problem in many scientific disciplines, our statistical paradigm should be applicable to other analytic procedures as well.

FY96 Publications, Presentations, and Other Accomplishments:

Ballantyne, J.C. and Brown, E.N. Latex anaphylaxis: Another case, another cause. Anesth. & Analg., 81, 1303-1304 (1995).

Brown, E.N. "Comments on 'Hierarchical models for ranking and for identifying extremes, with applications' by Morris, C.N. and Christiansen, C.L. in "Bayesian Statistics 5." Edited by: Bernardo, J.M., Berger, J.O., David, A.P., and Smith, A.F.M. Oxford University Press, pp 277-296, (1996).

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Brown, E.N. (contributor) Journal Club, Department of Anesthesia & Critical Care, Massachusetts General Hospital, Boston, MA (1996).

Brown, E.N. (invited participant) Subcommittee on Detection Limits of the National Committee on Clinical Laboratory Standards, Atlanta, GA (1996).

Brown, E.N. (invited speaker) Bios' 96, International Society for Optical Engineering, Ultrasensitive, Biochemical Diagnostics, San Jose, CA (1996).

Brown, E.N. (invited speaker) The University of Valladolid, Valladolid, Spain (1996).

Brown, E.N. (invited speaker) NeuroMusculer Research Center, Boston University (1996).

Brown, E.N. (invited speaker) Laboratory of Developmental Chronobiology, Massachusetts General Hospital, Boston, MA (1996).

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The Biomechanics of Exercise Countermeasures

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Funding:

Project Identification: 199-26-17-11
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $99,786
Students Funded Under Research: 5

Task Description:

Space flight can lead to a significant bone loss and to muscle atrophy. To date, no effective countermeasure has been identified for either of these undesirable effects. There are strong indications, however, that exercise will form a crucial part of any protocol to minimize the adverse effects of space travel. It is hypothesized that an effective exercise regimen should elicit loads on the lower extremities and require muscle actions that resemble those encountered in 1-G.

The objectives of the proposed study are to use a ground-based simulator of zero gravity to define exercise countermeasures in terms of their similarity to 1-G loads and patterns of muscle activity. This will eventually lead (in a subsequent proposal) to a logically planned in-flight experiment in which the efficacy of the proposed exercise program is studied directly in terms of its effect on muscle and bone mass.

During this funding period, ground reaction forces, loads in the tethering harness, lower extremity joint movements, and electromyographic data have been collected on 16 subjects during walking and running in four experimental conditions. Normal overground locomotion and locomotion on a conventional treadmill were the two 1-G conditions, while the two conditions in the Zero-Gravity Locomotion System (ZLS) were locomotion with tethering springs attached only at the subjects' shoulders and locomotion with the tethering springs attached at the subjects' waists and shoulders. The electromyographic and joint kinematic data have been combined to determine the phases of isometric, concentric, and eccentric activations of the tibialis anterior, gastrocnemius, rectus femoris, vastus lateralis, biceps femoris, and gluteus maximus muscles.

These experiments have demonstrated that in the ZLS, subjects had a tendency to walk and run with their knees significantly more flexed than during the 1-G conditions. This has been called "Groucho running" by some scientists because of the resemblance to the gait of the late Groucho Marx. This pattern of walking and running is most likely an attempt to reduce the discomfort of the forces applied to the body by the harness, which must also be used in space to pull the astronaut back to the exercising surface. The maximum ground reaction force was significantly less in the ZLS conditions, although the rate that the initial ground reaction force was applied in the ZLS conditions was greater than in the 1-G conditions. The muscular activations of the tibialis anterior, gastrocnemius, rectus femoris, vastus lateralis were significantly greater in the ZLS conditions, although the activations of the biceps femoris and gluteus maximus were not affected by the experimental condition. The tension fluctuation in the tethering springs was 18% of body weight in the "shoulder springs only" condition and 36% of body weight in the "waist and shoulder springs" condition. It appears as if this fluctuation was

related to many of the biomechanical differences between the 1-G and ZLS conditions in the kinematic, ground reaction force, and electromyographic variables.

In order to provide the maximum benefits of treadmill exercise during space flight, more attention must be paid to the manner in which the subject is tethered to the treadmill. Future work will be aimed at minimizing the fluctuation in the tethering springs so as to more closely resemble the constant pull of gravity, and at minimizing the harness discomfort to lessen the subjects' desire to "Groucho" walk and run.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Orthostatic Intolerance - Short Flights

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-70-11-20

Solicitation: 93-OLMSA-07

Initial Funding Date: 1/95

Expiration: 1/97

FY 1996 Funding: $82,000

Students Funded Under Research: 0

Responsible NASA Center: JSC

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.

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Gravity in Human Oculomotor Control, Perception and Action

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Funding:

Project Identification: 199-16-12-40
Initial Funding Date: 10/94
FY 1996 Funding: $155,000

Solicitation: 93-OLMSA-07
Expiration: 9/97
Students Funded Under Research: 11

Responsible NASA Center: ARC

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.

Facility/Laboratory Additions.
The Two Axis Human Rotation Device (TAHRD) that was formerly used by Professor Sheldon Ebenholtz at the SUNY College of Optometry has been re-assembled, and is currently located in Dr. Cohen's laboratory in Building N-239 at Ames. Specifications for the device have been compiled and reviewed, and the TAHRD has been scheduled for human rating at Ames.

The miniature binocular goggle-mounted ISCAN infra-red video camera has been modified for use in various acceleration environments. This new camera weighs less than 0.5 ounces, and is being used to obtain more precise measures of oculomotor control under a variety of conditions.

Experiments Conducted.
Several research protocols were completed during FY96. HR160 at the 20-G Human Centrifuge Facility examined how G-perception is affected by prolonged (up to 1-hour) exposures to 2.0 Gz hypergravity. The results of this study were not unequivocal, and data analyses are still ongoing.

Research Protocol HRII-080 was run with thirty-two subjects during the summer and autumn of 1996. The overall study examined the reaction times of subjects who learned to discriminate between two images of human faces when the images were initially presented in an erect or an inverted retinal orientation. In subsequent testing, the images were rotated on the subjects' retinae at various angles from erect. Our data revealed that, if the images were initially learned in the erect orientation, reaction times increased as the images deviated from erect. In contrast, if the images were initially learned in the inverted orientation, there was no significant effect of stimulus orientation in subsequent trials. Additional studies along these lines are in process.

Dr. Li completed studies of human perception of the zenith (HRII-081) as it relates to oculomotor control. The study involved use of the ISCAN camera to record eye position when the orientations of subjects were changed and when they placed a visual target to the apparent zenith. These results have been submitted for presentation to the 1997 European Conference on Visual Perception.

Research Protocol HRII-082 examined the effects of stimulus orientation on subsequent recognition of airport runways when the runways are seen in various orientations. The study demonstrated that discriminative responses to maps of airports were most rapid when the maps were seen in the same orientation as that in which they were initially learned, that reaction time was reduced with repeated stimulus exposures, and that information learned from navigation maps was not sufficient for all observers to recognize aerial photographs of the same airports. These results have been submitted for publication in Aviation, Space and Environmental Medicine.

Data collection on Protocol HRII-097 was completed in September of 1996. This experiment examined the effects of orientation on the ability of subjects to recognize facial effect. The results of the study have been accepted for presentation at the 1997 annual scientific meetings of the Aerospace Medical Association.

The current research is expected to increase our understanding of how gravity combines with visual stimulation to influence oculomotor control, perception, and visual-motor behavior, both on Earth and in space. This information is important in understanding human spatial orientation and disorientation, as well as how intersensory interactions and feedback mechanisms operate to modify perceptual and behavioral functioning. The development of analytic, descriptive, and predictive techniques will enhance our understanding of the underlying mechanisms that operate in both terrestrial and space environments and under both normal and abnormal physiological conditions. To the degree that our models can be used to describe normal physiological and behavioral capabilities, they can also be used to determine and to quantify deficits in behavior that result from disease states. Finally, these studies are potentially useful in showing how spatially-coded information can best be presented to individuals so that their learning is optimized.

FY96 Publications, Presentations, and Other Accomplishments:

Cohen, M.M. Elevator illusion and gaze direction in hypergravity. Av., Space, & Environ. Med., 67, 676 (1996).

Stoper, A.E., Randle, J., and Cohen, M.M. The effect of environmental pitch on perceived optic slant and eye level: Lines vs. dots. 19th European Conference on Visual Perception, Strasbourg, France, September 7, 1996.

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NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis

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Project Identification: 199-18-17-13
Solicitation:

Initial Funding Date: 1/94
Expiration: 1/97

FY 1996 Funding: $388,259
Students Funded Under Research: 12

Task Description:

The NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis is dedicated to the application of quantitative methods to understand the basic mechanisms involved in alterations in cardiovascular functions during and after space flight. As part of this effort the Center develops new technologies to measure alterations in cardiovascular function and to guide the application of possible countermeasures. Many of these technologies are useful not only in the context of space flight research and space medicine but also have important spin-off applications for clinical medicine on Earth. In fact, a number of technologies developed in the Center are in the process of being commercialized for clinical application. One of these spin-off technologies—a non-invasive diagnostic test for identifying individuals at risk for ventricular arrhythmias and sudden cardiac death—has received FDA approval and is being marketed in the United States, Japan, and Europe. In addition to its research function, the NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis has training and educational components. The Center is involved in training undergraduate students, graduate students, and postdoctoral fellows to conduct research in quantitative cardiovascular space physiology. In addition, the Center sponsors a number of educational activities to expose to this field students, university investigators, and scientists and engineers in government and industry.

The NASA Center for Quantitative Cardiovascular Physiology, Modeling and Data Analysis received its initial funding in early 1994 (official start date 1/15/94; funding received circa 5/94). Over this two-year period, the Center has successfully assembled an international staff with a broad range of backgrounds and established its research and training programs. The Center has appointed a distinguished external advisory board to help guide the principal investigator in directing the Center. The Center's research program has already resulted in over 30 published articles. The Center sponsors a quantitative cardiovascular course for medical students and Ph.D. students within the HST Division as well as a parallel summer course which draws scientists and engineers world-wide from academia, government, and industry. Both of these courses are directed by the principal investigator, Richard J. Cohen. In addition the Center has held two annual symposia presenting its research activities. This past year, the General Electric Company has decided to establish an ultrasound research program in conjunction with the Center. It has granted the Center its most modern equipment together with a proprietary computer interface so that investigators at the Center can directly access and process the raw ultrasound signals with a personal computer.

Cardiovascular System Identification

The intact cardiovascular system maintains arterial blood pressure (ABP) within a fairly narrow range despite a wide variety of physiological perturbations. Such perturbations may include, for example, respiratory effects on venous return and the filling of intrathoracic cardiovascular structures, the fluctuations in peripheral vascular resistance as tissue beds adjust local vascular resistance in order to match local blood flow to demand, changes in body posture, and physiological stresses such as exercise and variations in environmental temperature. These perturbations to cardiovascular homeostasis are compensated for by a variety of cardiovascular control mechanisms such as the arterial baroreflex and the Bainbridge reflex. Homeodynamics involves the dynamic processes by which physiological control mechanisms maintain physiological variables in a limited range over time in the face of time varying perturbations. Although the basic mechanisms involved in short term cardiovascular regulation have been elucidated, their integrated homeodynamic functioning remains poorly understood.

Mean values of physiological variables provide little insight into homeodynamic mechanisms. However, the study of the beat-to-beat fluctuations in cardiovascular variables do provide a means for assessing the integrity of closed-loop hemodynamic regulation. In the Center, we have developed a new quantitative methodology for the non-invasive assessment of closed-loop cardiovascular regulation by analysis of the interrelationship between second-to-second fluctuations in multiple hemodynamic and other physiologic signals. The fluctuations in the various signals are analyzed using a parametric system identification methodology involving an autoregressive moving average (ARMA) set of equations to describe the causal couplings between the signals (CSI).

CSI is a very powerful methodology. It involves a kind of 'inverse modeling' that takes epochs of physiological data and creates from them an individualized regulatory model for the specific subject being studied. The complexity of the model 'identified' using system identification methods depends on the number of physiological signals being analyzed. In principle, when n signals are analyzed, one may characterize n(n - 1) causal couplings and n noise sources. When more signals are analyzed, one may dissect the couplings and noise sources into more constituent components.

In the cardiovascular system identification model that we have used for the identification of the couplings between fluctuations in heart rate, ABP, and instantaneous lung volume (ILV), the fluctuations in these variables are interrelated through five coupling mechanisms: CIRCULATORY MECHANICS, HR BAROREFLEX, SA NODE, ILV*HR, and ILV*ABP.

In addition to the five coupling mechanisms, the model incorporates two perturbing noise sources, NHR and NABP. NHR represents the fluctuations in HR not caused by fluctuations in ABP or ILV. Such fluctuations may result, for example, from autonomically mediated perturbations driven by cerebral activity. NABP represents fluctuations in ABP not caused by fluctuations in heart rate or ILV. Such blood pressure fluctuations may result, for example, from fluctuations in peripheral vascular resistance as tissue beds adjust local vascular resistance in order to match local blood flow to demand or from beat-to-beat fluctuations in stroke volume.

The perturbations represented by NHR and NABP as well as the variability in ILV are responsible for driving all fluctuations in HR and ABP through the coupling mechanisms. NHR and NABP are not directly measured quantities. They represent the residual variability in HR and ABP once one subtracts out the components of variability caused by the fluctuations in each case in the other two measured signals.

In order to completely identify the CSI model, six minute epochs of ECG, ILV measured using a noninvasive Respitrace system (Ambulatory Monitoring Systems, Inc.), and intra-arterial ABP are obtained. Data is collected during a random interval breathing protocol during which subjects breathe in response to auditory cues at a comfortable mean rate of 12 breaths per minute but with inter-breath intervals randomly varying between one and 15 seconds. Subjects adjust their own tidal volumes thereby leaving blood gases unperturbed. The random interval breathing protocol broadens the frequency content of the recorded physiological signals, thereby facilitating CSI. Using this data, each coupling (except for SA NODE which is a predefined, nonlinear

"integrate and fire" device) and perturbing noise source power spectrum may be identified from a pair of linear, time-invariant ARMA difference equations. Each coupling element is represented in terms of its estimated impulse response function - the response of the system to a transitory perturbation of arbitrarily short duration. The noise sources are represented in terms of their power spectra (energy as a function of frequency).

During combined β-sympathetic and parasympathetic blockade, the amplitude of the autonomically mediated physiological coupling mechanisms (HR BAROREFLEX and ILV→HR) are both reduced essentially to zero as is the amplitude of the power spectrum of NHR which represents autonomically mediated perturbations to heart rate. Conversely, the amplitude of the mechanically mediated couplings (ILV→ABP and CIRCULATORY MECHANICS) are preserved. There is a partial diminution in the power spectrum of NABP. The remaining power in NABP may reflect non-autonomically mediated perturbations to ABP resulting perhaps from local autoregulatory fluctuations in peripheral vascular resistance. There is also a reduction in amplitude of fluctuations in ILV representing decreased tidal volume perhaps due to β-sympathetic mediated bronchio-constriction.

Our results demonstrate that CSI correctly predicts the effect of pharmacological blockade on those couplings and noise sources which are autonomically mediated. Our desire is to be able to apply CSI to the study of chronic changes in cardiovascular regulation which occur during and after space flight. As a ground-based model of chronic changes in autonomic function in man, we chose to study patients with diabetic autonomic neuropathy. Diabetic autonomic neuropathy represents a progressive deterioration in sympathetic and parasympathetic nervous system function in patients with diabetes. Diabetic autonomic neuropathy is related to the inadequacy of glucose control, and noninvasive monitoring of this condition is important as a physiological guide to the management of diabetes. We studied 60 diabetic subjects and 37 control subjects. The diabetic subjects were divided into three groups with minimal, moderate, and severe autonomic neuropathy on the basis of standard autonomic testing. The minimal group was indistinguishable from the control group on the basis of the conventional testing. CSI was performed totally non-invasively in these subjects with ABP recorded using a Finapres non-invasive transducer (Ohmeda, Inc.). CSI results, corrected for patient age, revealed a progressive diminution in the autonomically mediated physiological coupling mechanisms with increasing degree of autonomic neuropathy across the four groups, while the mechanically mediated couplings were not affected. Interestingly, the CSI results revealed a statistically significant difference in autonomic function between the control and minimal groups which was not revealed by standard autonomic testing. This study demonstrated in this ground-based model that progressive changes in autonomic function could be quantitatively assessed by CSI methods.

Non-invasive Measures of Susceptibility to Ventricular Arrhythmias

While the effects of space flight on cardiac conduction processes are not known, it is thought that a variety of the physiological stresses occurring during space flight, such as prolonged exposure to microgravity, high G forces, and hypoxia, may affect cardiac conduction processes. A variety of heart rhythm disturbances have been documented in astronauts and in pilots during flight, during exposure to high G forces in centrifuge studies, and exposure to lower body negative pressure. These heart rhythm disturbances have included such potentially serious arrhythmias as ventricular tachycardia. In the civilian population, there are approximately 300,000 sudden cardiac deaths annually in the United States alone. The proximate cause of death in the vast majority of these cases is ventricular fibrillation. Sustained ventricular tachycardia degenerating into ventricular fibrillation may have occurred in many of these cases. It is likely that ventricular arrhythmias during space flight will be of increasing concern in the future as the durations of missions lengthen and as older individuals are involved in space flight. Older individuals have a greater statistical likelihood of having underlying structural heart disease, in particular coronary artery disease, and thus will be at greater risk for heart rhythm disturbances.

The Center has been involved in an effort to understand quantitatively the basic mechanisms leading to re-entrant ventricular arrhythmias and to develop new techniques to non-invasively assess an individual's risk for spontaneous ventricular tachycardia or fibrillation. The investigation of basic mechanisms is necessary to understand how space flight may lead to cardiac electrical instability, what countermeasures are likely to be

effective, and to develop new non-invasive technologies for monitoring an individual's susceptibility to cardiac arrhythmias. The development of new non-invasive technology for assessment of risk of ventricular arrhythmias is urgently needed in order to be able to study experimentally the effects of space flight on cardiac electrical stability, to identify individuals potentially at risk of arrhythmias during space flight, and to evaluate experimentally of possible countermeasures. Development of such non-invasive risk assessment techniques also has important spin-off applications to civilian medicine. Although effective treatment is currently available to prevent sudden cardiac death by means of the implantable cardioverter defibrillator, only a tiny fraction of sudden deaths are currently prevented. This is because it is not known who is at risk; sudden death is the very first manifestation of heart disease in one-third of the cases. There is a great need to have an accurate non-invasive means of screening individuals to identify those at risk of life-threatening ventricular arrhythmias.

In previous work, we studied the development of re-entrant arrhythmias in a finite element computer model of cardiac conduction processes incorporating spatial dispersion of refractoriness. This finite element computer model displayed a variety of re-entrant rhythm disturbances including non-sustained and sustained ventricular tachycardia and ventricular fibrillation. We also observed that under conditions when this computer model displayed a susceptibility to reentrant ventricular arrhythmias, that the 'normal' beats displayed an alternating morphology in successive beats. This pattern is known clinically as 'electrical alternans.' In this computer model re-entry occurs because some of the elements in the model have a refractory period longer than the interbeat interval. This leads to incoming depolarization wavefronts fractionating and re-entering upon encountering islands of refractory tissue. The fact that refractoriness is prolonged in some regions of tissue means that different regions do not conduct, or do not conduct normally, on alternate beats. This process will lead to alternation in conduction patterns. Thus, the underlying dispersion of refractoriness leads both to the development of re-entry on the one hand and to alternation on the other hand.

Electrical alternans is a very rare clinical finding. (Electrocardiographic alternans can be seen in the setting of pericardial effusion. In this case, the alternans is a result of the entire heart mechanically alternating its motion leading to rotation of the electrical axis of the heart and not a result of alternation of electrical conduction processes in the heart. We do not consider such electrocardiographic alternans here.) We hypothesized that very subtle electrical alternans in which the morphology of electrocardiographic complexes varied only by microvolt amounts from one beat to the next may be present in individuals at risk for ventricular arrhythmias. We developed a power spectral method of detecting such microvolt levels of electrical alternans from analysis of 128 consecutive ECG complexes.

In animal studies, we showed that the presence of T wave alternans correlated with enhanced susceptibility to ventricular arrhythmias as measured by the ventricular fibrillation threshold during a variety of different interventions including systemic hypothermia, coronary artery ligation, and tachycardia. We show the inverse relationship between the ventricular fibrillation threshold and the level of electrical alternans in 120 paired measurements made on 20 dogs. These data show that the relationship between T wave alternans and cardiac electrical stability holds regardless of the intervention used to alter susceptibility.

Subsequently, we conducted a study in patients undergoing invasive electrophysiologic testing at the Massachusetts General Hospital. Invasive electrophysiologic testing (EP) is a procedure used in patients thought to be at high risk of ventricular arrhythmias. During EP, catheter electrodes are placed in the patient's heart, and a deliberate attempt is made to induce ventricular arrhythmias in order to determine a patient's susceptibility. In this study, prior to EP, we recorded ECG signals during atrial pacing and analyzed the signals for the presence of alternans. We compared the presence of significant levels of alternans with the outcome of EP and 20 month arrhythmia-free survival. Patients without alternans had approximately a 95% rate of arrhythmia free survival, whereas the patients with significant levels of alternans had only a 20% rate of arrhythmia free survival. In this study, the presence of electrical alternans was equivalent to EP as a predictor of arrhythmia-free survival. Measurement of T wave electrical alternans is a non-invasive test, whereas EP is highly invasive, expensive, and not risk-free. We found that signal averaged ECG, the presence of ventricular premature beats, and QT dispersion (other measures which have been used for assessment of risk of ventricular arrhythmic events) were not useful predictors of arrhythmic events in the population we studied.

In order for electrical alternans to be a sensitive measure of susceptibility to ventricular arrhythmias, the heart rate must be elevated. In the clinical study described above, heart rate was elevated by means of atrial pacing. In order for measurement of electrical alternans to be a fully non-invasive measure, heart rate must be elevated using non-invasive means. To accomplish this task, MIT licensed the alternans technology to a startup company, Cambridge Heart Inc., which has now successfully developed instrumentation for measuring electrical alternans during bicycle exercise. In 26 patients studied in collaboration with Dr. Mark Estes, exercise-induced alternans was a highly accurate predictor of inducibility of sustained ventricular tachycardia or fibrillation during EP testing (sensitivity = 100% and specificity = 86%).

Cambridge Heart Inc. has obtained FDA approval to market the instrumentation to measure T wave alternans at rest and during exercise stress testing. In a series of clinical protocols, approximately 25 Centers around the world are now evaluating the measurement of T wave alternans as a predictor of arrhythmic events and sudden death in many different patient populations. There is great excitement in the cardiac electrophysiology community that measurement of T wave alternans may be the powerful non-invasive predictor for arrhythmic events which will enable physicians to identify and treat (e.g., by implantation of the internal cardioverter/defibrillator) many of the 300,000 individuals who die annually of sudden cardiac death. The development of the technology to non-invasively measure T wave alternans now provides us with a tool to evaluate the effect of prolonged exposure to microgravity on cardiac electrical stability.

Non-Invasive Imaging of Cardiac Electrical Activity

Because the electrical activity of the heart is so intimately related to cardiac function, nearly all alterations in cardiac function also lead to alterations in cardiac conduction processes. For example, cardiac ischemia, chamber enlargement and/or hypertrophy, and heart rhythm disturbances all lead to changes in cardiac electrical activity. However, the standard ECG provides very limited information on cardiac electrical activity. In particular, the standard ECG provides essentially no information on the spatial distribution of cardiac electrical activity in the heart. In order to study non-invasively the effects of space flight on cardiac function, it is desirable to be able to non-invasively map or image cardiac electrical activity. Our laboratory has developed a technique for accomplishing this task by measuring the second spatial derivative (the surface Laplacian) of the body surface potential with an array of concentric disk bipolar electrodes. Each of these electrodes records the electrical activity only from the region of myocardium directly underneath the electrode. Because the recording is so highly localized, it is possible to construct, at each instant in time, a projection image of the distribution of cardiac electrical activity. With this method, for example, right atrial activity is restricted only to the electrodes immediately overlying the right atrium, whereas when mapping body surface potential activity right atrial activity is broadly distributed over the thorax.

We have evaluated the ability of the surface Laplacian method to localize myocardial ischemia during coronary angioplasty. In eleven patients undergoing coronary angioplasty, we recorded the 12 lead standard ECG, the body surface potential map at 84 precordial locations, and the body surface Laplacian map at the same 84 precordial sites. We calculated the change in ST segment level divided by baseline QRS amplitude ($\Delta ST/Q$) as a measure of ischemia during the period of balloon inflation. We defined $\Delta ST/Q > 0.1$ to be significant. The 12 lead ECG revealed a significant change in only four of 11 patients. The body surface potential map using unipolar electrodes revealed a significant change in $\Delta ST/Q$ in eight of 11 patients but correctly localized the change to the correct anatomical location in only one of 11 patients. The body surface Laplacian revealed significant ischemic changes in the correct anatomic locations in all 11 patients. This demonstrates that body surface Laplacian mapping may provide an accurate and sensitive means of localizing changes in cardiac electrical activity during space flight. Laplacian mapping may be applied, for example, to study the effects of space flight on myocardial perfusion. This may be very important particularly as older individuals are engaged in space flight. These older individuals will have a greater probability of having underlying 'silent' coronary artery disease. In addition, non-invasive means of imaging myocardial ischemia, particularly during exercise stress, would be a very important spin-off technology for clinical medicine. Currently, very expensive radio-isotope methods are utilized for non-invasive imaging of cardiac ischemia.

Finite Element Modeling of Cardiac Electrical Activity

The development of both microvolt level electrical alternans as a measure of cardiac electrical stability and body surface Laplacian imaging of cardiac electrical activity in our laboratory was based on our finite element models of cardiac electrical activity. We have been improving our electrophysiologic models so as to be able to more accurately simulate the development of arrhythmias and surface ECG activity. We have been particularly interested in the relationship between the spatial dispersion of cardiac recovery and the development of reentrant arrhythmias such as fibrillation. We have shown that in order for finite element models of cardiac electrical activity to be physiologically accurate, the parameters of the finite element model are constrained. The propagation velocity must vary proportionately with wave-front curvature with the proportionality coefficient being an effective 'diffusion constant' which depends only on the passive membrane properties. Also, the spatial location of the finite elements in the model must be randomized in order to prevent a regular lattice structure from artifactually dominating the behavior of the model. We have shown that a number of models published in the literature which demonstrate fibrillatory break-down in normal tissue, become stable when the models are corrected to take the above constraints into effect.

EDUCATIONAL AND TRAINING ACTIVITIES

Five graduate students, five postdoctoral fellows, and one research staff member are engaged full time in the activities of the Center (see Appendix for listing of Center staff and Advisory Board). In addition, undergraduates, Vivian Jung (Wellesley) and Linda Rosenband (MIT) devoted 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 Ph.D. 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 and includes aspects of space cardiovascular physiology. Also, the Center sponsored its first symposium on November 17, 1994, and its second, on January 18, 1996. In addition, the Center has made provision for scientists to visit for periods of varying length in order to promote collaboration. This past year we have hosted visits by Drs. Solange Akselrod and Josef Starobin.

The Center plans to continue all of the above efforts, integrating undergraduate and graduate students as well as postdoctoral fellows into its programs. In addition, the Center will continue to educate students and professional scientists and engineers from academia, government, and industry in the area of quantitative cardiovascular physiology as applied to space flight through the courses it sponsors and its symposia.

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; and

3) Noninvasive imaging of cardiac electrical activity.

FY96 Publications, Presentations, and Other Accomplishments:

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Effects of Acute Intense Exercise and Microgravity on Mechanisms Associated with Blood Pressure Regulation in Humans

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Project Identification: 199-14-17-01
Initial Funding Date: 2/95
FY 1996 Funding: $ 104,100

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 4

Task Description:

Reductions in plasma volume (hypovolemia relative to 1-G) and autonomic dysfunction in humans have been documented following exposure to actual and ground-based simulations of microgravity. Since these cardiovascular adaptations are associated with post-space flight orthostatic hypotension, partial or complete restoration of microgravity-induced alterations in vascular volume and autonomic functions should therefore enhance orthostatic stability and contribute to the safe return and rapid recovery of crew members. Cycle ergometry exercise designed to elicit maximal effort has been successfully used to increase plasma volume and carotid baroreflex sensitivity in ambulatory subjects. Therefore, the purpose of this investigation is to test the hypothesis that a single bout of cycle exercise designed to restore plasma volume and reverse autonomic dysfunction within 24 hr of reambulation following exposure to simulated microgravity can ameliorate orthostatic hypotension and intolerance. A three-year human physiology research project is presented in this proposal which is designed to: (a) describe dynamic changes in blood volume, hormone responses, autonomic functions, and hemodynamic responses to orthostatic hypotension induced by exposure to an analog of microgravity; (b) describe interactions of these systems with each other; and (c) test the responses of these systems during the 24-h recovery period following acute intense exercise. The study will be conducted using 16 days of 6° head-down tilt (HDT) to determine the effects of extended duration exposure to microgravity on mechanisms that contribute to blood pressure control and if the restoration of these mechanisms will reverse orthostatic intolerance. Plasma volume, leg compliance cardiopulmonary and arterial baroreflex functions, adrenoreceptor function, cardiac and hemodynamic measurements, and vasoactive hormone responses will be measured in subjects before and after HDT with and without exercise treatment to determine the effect of reversing altered mechanisms associated with blood pressure regulation on orthostatic tolerance. Our expected result from this investigation is that a single exposure to acute exercise designed to elicit maximal effort within 24 hr of reambulation from HDT will provide a stimulus that reverses hypovolemia and autonomic dysfunctions induced by microgravity and eliminate orthostatic intolerance. Results of these studies should provide a better understanding of the adaptive process of components of the blood pressure control system during recovery from acute exercise and to microgravity environments, and a physiological basis for development of specific effective countermeasures against orthostatic hypotension following space flight.

We have completed the exposure of seven subjects to 16 days of 6° head-down tilt under two experimental conditions: 1) with application of a single exposure of cycle ergometer exercise designed to elicit maximal effort within 24 hours of reambulation; and 2) without exercise (control). With these data, we have been able to demonstrate that a single bout of maximal exercise was effective in ameliorating the adverse effects of hypovolemia, reduced adrenergic responsiveness, limited peripheral vascular constriction, and attenuated carotid cardiac baroreceptor responsiveness on orthostatic stability caused by exposure to a ground-based analog of microgravity. Our results provide new insight into the potential use of acute intense exercise to reverse detrimental effects of adaptation to microgravity on cardiovascular mechanisms associated with blood pressure regulation and orthostatic performance following spaceflight. Our results may suggest that acute intense exercise increases the responsiveness of cardiac and vascular adrenergic receptors as well as cardiopulmonary and aortic baroreflexes. We plan to investigate these new hypotheses in the coming year.

Results from our experiments should provide new understanding of mechanisms underlying the clinical condition of orthostatic hypotension, from patients who are restricted to prolonged bedrest or with autonomic dysfunctions to astronauts following a space mission. The results from the testing of acute intense exercise proposed in this research can provide a new potential therapeutic for acute management of orthostatic hypotension and intolerance. We have already implemented the use of this protocol to eliminate orthostatic hypotension in a group of paraplegic patients. The results of this research could provide a simple technique to help alleviate clinical symptoms associated with orthostatic hypotension.

FY96 Publications, Presentations, and Other Accomplishments:

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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
Initial Funding Date: 6/94
FY 1996 Funding: $112,000
Joint Agency Participation: DoD (USAF)

Solicitation:
Expiration: 6/97
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 bedrest studies. The primary objective of this research is to invasively measure specific hemodynamic responses in a non-human primate model during exposure to 10 degrees head-down tilt (HDT), a surrogate of microgravity, in order to test two hypotheses that may explain mechanism(s) of decreased CVP in the face of maintained/increased cardiac output caused by space flight: 1) that there is an increase in cardiac compliance associated with exposure to microgravity, and/or 2) that there is a resetting of the CVP set-point to a lower operating range. Hemodynamic and adrenergic data will be obtained from ten chronically-instrumented rhesus monkeys. The test protocol consists of five days exposure to 10 degree head-down tilt (treatment condition) and five days of 80 degree head-up tilt (control condition) separated by one week of return to baseline in a cross-over counterbalance design. Hemodynamic measurements will include pressures of the left ventricular, right atrium, aorta, and esophagus, aortic flow, cardiac output, cardiac chamber areas (transesophageal echocardiography), hormone levels, and plasma volume. Provocative test measurements will include Dextran infusion, phenylephrine infusion (alpha-receptor sensitivity), isoproterenol infusion (beta-receptor sensitivity), and lower body positive and negative pressure. Identifying mechanisms

underlying the reduction in CVP in microgravity could prove instrumental to the development of effective countermeasures against orthostatic hypotension induced by both G-layoff or space flight.

We measured specific hemodynamic responses during 4 days of head-down tilt in invasively-instrumented rhesus monkeys to test the hypothesis that exposure to simulated microgravity causes increased cardiac compliance. We have successfully completed experimental testing of all seven subjects, have analyzed their results, and remain on schedule to complete writing of manuscripts and reports by May 1997. Rhesus monkeys underwent the following two 5-day experimental conditions separated by 9 days of return to baseline ambulatory activities in a cross-over counterbalance design: 1) continuous exposure to 10° HDT; and 2) 16 hours per day of 80° head-up tilt and 8 hours supine (control). Each animal underwent daily baseline measurements of central venous pressure, left ventricular (LVP) and aortic (AoP) pressures, stroke volume (SV), and esophageal pressure (EsP). Additionally, each animal underwent measurement of plasma volume (HDT day 2) and provocative tests which included graded dose administration of phenylephrine (a1-adrenergic responsiveness) and isoproterenol (b-adrenergic responsiveness) (HDT day 3), and application of lower body negative pressure (HDT day 4). Compared to the control condition, HDT reduced CVP by 34% (1.6 mmHg, $P = 0.010$), but stroke volume was reduced by only 17% ($P = 0.094$). The proportionately greater reduction in CVP compared to stroke volume during HDT was associated with increased mean left ventricular end-diastolic compliance of 0.894 ± 0.143 ml/mmHg compared to 1.111 ± 0.170 ml/mmHg in the control condition ($P = 0.0053$). Increased cardiac compliance could not be explained by reduced thoracic transmural pressure since EsP was unaltered by HDT. Our data provide the first direct evidence that increased cardiac compliance is associated with headward fluid shifts similar to those induced by exposure to microgravity, a finding consistent with the observations in human space flight.

The results of this study have also provided evidence that the CVP operational point (setpoint) and the renal urine excretion to fluid loading are reduced with the HDT condition. These data are consistent with observations made during spaceflight on astronauts and raise the possibility that the sensitivity of receptors in the kidney to hormones that regulate fluid homeostasis may be altered with exposure to microgravity. These alterations in CVP operational point and renal function may have significant consequences for limiting fluid replacement prior to return from space flight and subsequent attempts to develop effective countermeasures. We plan to use our primate HDT model and pharmacological interventions to examine possible changes in renal mechanisms associated with body fluid homeostasis during exposure to a gound-based analog of microgravity.

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 pressure procedures. A review of literature indicated that ketamine 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 at least three minutes. In addition, we have observed elevations in aortic, left ventricular, and right atrial pressures and aortic flow following insertion of the TEE probe. It remains uncertain, however, whether TEE insertion alters baroreflex response. We have conducted an experiment to determine the effects of ketamine and TEE insertion on cardiovascular function and baroreflex responsiveness. 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 bedrest. This knowledge could be instrumental in the development of therapeutic management for dehydration effects in patients with restricted physical activity as well as with astronauts following a space mission. These mechanisms could contribute to the orthostatic hypotension and intolerance experienced by both patients and astronauts. Our results will also provide some new insight into the cardiovascular effects of ketamine, a human pediatric anesthetic. If reduced CVP setpoint proves to be an adaptation in these experiments, this could provide a basis for development of new therapeutic techniques

designed to acutely increase the CVP setpoint to enhance vascular volume, cardiac filling pressure, and consequently, defend blood pressure regulation during orthostatic challenges.

FY96 Publications, Presentations, and Other Accomplishments:

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Blood Volume Regulation in Primates During Space Flight

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Project Identification: 199-14-17-12
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/97
FY 1996 Funding: $ 159,914
Students Funded Under Research: 2

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.

The objectives of the study were: 1) Develop a non-human primate model that simulates the cardiovascular deconditioning that is exhibited by the astronauts; 2) Determine how in the baroreflex control of blood pressure is altered during and after 72 hrs of simulated microgravity; 3) Determine if there is an alteration in the control of blood volume during simulated microgravity; and 4) Investigate possible countermeasures that could be used in order to prevent the orthostatic hypotension that has been observed in the astronauts.

This study has involved the use of eight chronically instrumented Rhesus monkeys. Cardiovascular instrumentation included arterial and venous catheters and aortic and renal Doppler flow probes. After training the animals to the restraint chair, they were placed in a water tight suit and then were immersed in the upright position to the level of the mid chest. The immersion period lasted for 72 hrs and was preceded and followed by two hours of control. The animals tolerated this well and ate and drank during the procedures. Renal and

cardiovascular data were recorded continually and urine was collected hourly. The psychological well-being of the animal was ensured by having someone with them continually as well as being provided with soft music and a variety of fruits. The animals tolerated this very well and interacted with the technician.

The control immersion consisted of 72 hrs with no additional volume supplement. Catheter lines were maintained patent by infusing lactated Ringer's solution at a rate of 3 ml/hr/catheter. The baroreflex was determined before immersion, four hours into the immersion and then at 28, 52, and 70 hrs of immersion and then after the immersion. The first intervention was to maintain fluid balance by infusing 51 ml lactated Ringer's solution/hr over the entire study period. Alterations in the control of blood volume were determined by volume expanding the animals with 6% dextran in normal saline (this is isotonic isoncotic). The volume expansion was done as a control on a day other than the immersion day and then again on the last day of the immersion. This was done with and without the maintenance of fluid volume.

Results:

Control study.

1. There was an increase in blood pressure and CVP during the immersion which gradually return towards control by the end of the immersion. Blood pressure and CVP returned to below controls when removed from the tank. This was associated with a decrease in heart rate during the immersion. After the immersion the heart rate was significantly elevated above pre-immersion levels.

2. There is a shift in the baroreflex curve upwards and slightly to the right after immersion.

3. The animals become gradually hypotensive during the first hr after immersion.

4. The animals went into a significant negative water balance early in the immersion and remained so throughout the immersion. The animals become adipsic and need to be encouraged to drink.

5. There was an initial increase in ANF during the immersion which returned to normal or below after 24 hrs of immersion. It increases above control levels after the immersion, probably due to the tachycardia.

6. There was a slight increase in urine output within the first two hours of immersion that continued throughout the immersion. This was associated with a similar increase in sodium excretion.

7. The blood pressure was very sensitive to the administration of nitroprusside and relatively insensitive to phenylepherine.

Immersion with maintenance fluid volume.

In this protocol the hydration level was established for three hours before the immersion by infusing 50 ml/min lactated Ringer's solution. This is continued throughout the immersion. This intervention expands both the vascular and extravascular spaces.

1. The Hemodynamics were similar to those noted above. The CVP increases to a greater degree than without the infusion. After the immersion the blood pressure tended to be at or above the pre-immersion control levels.

2. The baroreflex still shifted to the right but was more sensitive. There is a decreased hypotension with nitroprusside. There is no tendency for the animals to become hypotensive during the post-immersion period but significant tachycardia was still exhibited.

3. The animals still went into a negative water balance; however, not to the same degree as without the infusion.

4. The alterations in ANF are similar to the control condition.

5. The diuresis and natriuresis were greater and were maintained throughout the immersion.

Immersion with VE and no infusion.

This intervention was intended to determine the sensitivity of the volume control mechanisms. It also provided information on the effects of filling the vascular space on the control of blood pressure post immersion.

1. A volume expansion with isoncotic isotonic dextran during the last six hours of immersion did not cause a significant diuresis.

2. The baroreflex curve was shifted upwards with the same saturation. However there was still tachycardia as seen in the control immersions.

3. The animals did not become hypotensive to any degree during post immersion period. They were also less sensitive to nitroprusside and more responsive to phenylepherine.

4. The animals were in a negative water balance at the end of the immersion.

Volume expansion with infusion.

The volume expansion was intended to test the reflex control of blood volume during the immersion. It also replenished both the depleted vascular volume as well as the extracellular volume that has been lost during the immersion.

1. There was a significant diuresis in response to the dextran infusion given just before de-immersion. In some instances this exceeded the volume given during the expansion.

2. The baroreflex curve shifts upwards and to the right.

3. There is a decreased tachycardia post-immersion.

4. Some of the animals were in a negative fluid balance post-immersion.

5. The animals were relatively insensitive to nitroprusside post-immersion and very sensitive to phenylepherine.

Conclusions:

Water immersion of the non-human primate to the level of the mid chest simulates the cardiovascular changes reported during microgravity in astronauts. Our results would suggest that there is a diuresis during exposure to microgravity. The degree is related to the level of hydration of the astronaut. This diuresis would probably be most evident early in the flight. Volume maintenance before and during microgravity may actually increase the diuresis but also decreases the degree of post-flight orthostatic hypotension. It would appear that the control of blood volume is enhanced during exposure to microgravity. Therefore volume loading immediately prior to re-entry may actually enhance the diuresis associate with increased fluid volume. However, it still reduces the post-flight hypotension. As would be expected the sensitivity to interventions that produce hypotension is decreased by restoring vascular and extra vascular volumes. None of these interventions completely restore baroreflex post-immersion.

There are several conditions which cause alterations in the control of blood pressure and fluid volume. The changes reported here in alterations associated with the baroreflex during simulated microgravity are similar to

those reported with chronic congestive heart failure. However the alterations observed in the reflex control of blood volume are not consistent with those seen in heart failure. It may be that there are similarities with other disease conditions that represent states of relative hypovolemia.

In many regards the observation of post flight orthostatic intolerance are similar to the orthostatic intolerance reported in man. Under these conditions there appears to be both a volume component and a cardiovascular reflex component. Most of the interventions are directed at the changed reflex component. This study would suggest that adequate volume control may prevent the severity of the orthostatic hypotension even though it doesn't restore the altered reflex component.

FY96 Publications, Presentations, and Other Accomplishments:

Cornish, K.G., Werth, A.D., and Dreessin, A. Upright water immersion of the non human primate as a model of cardiovascular adaptation to micro gravity. FASEB Journal, 10(3), A658 (1996).

Posture Load-Induced Bone Maintenance: A New Hypothesis (split with Frangos)

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Project Identification: 199-26-17-04
Initial Funding Date: 1/93
FY 1996 Funding: $80,000

Solicitation:
Expiration: 1/96
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 Zeng [1,2], 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 two to three times as large as the shear stresses due to locomotion and thus might be the principal stimulus for bone growth.

We have established a theoretical basis for the mechanism by which small strain magnitudes at higher frequencies are capable of maintaining bone as well as larger strains at lower frequencies. Previously it had been

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. The hypothesis and model we proposed 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 has been verified with animal experiments at Stony Brook. 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 same order (10-20 dynes/cm2) as the shear stresses on the endothelium in the vascular system. Frangos (our grant is split with Frangos) and coworkers have demonstrated that cAMP, IP3, PGE2 production are elevated in osteoblasts subjected to such shear stresses. 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. The opening and closing of these communicating junctions have been demonstrated in many other cell types to be regulated by second messengers or transjunctional voltage. This leads to a change in intracellular potential between cells, the electrical excitation signal.

In the final year of this grant our major focus has been on cell to cell communication of osteocytes. We have constructed a model for electrical signal transmission and gap junction regulation in bone cell network. We have applied this cable model to an osteon and demonstrated the physical parameters that control this high frequency mechanism for intercellular signaling. Our first model lumped the gap junction resistance into one parameter; we have refined the model to show the effect of the discrete gap junction. This method of intracellular signaling is now well enough understood to begin an experimental investigation. We have engaged Dr. David Spray of the Albert Einstein School of Medicine, a world class authority on gap junctions, to initiate a joint experimental program to verify our model of electrical signal transmission and gap junction regulation in bone cell networks.

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 two to three 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.

FY96 Publications, Presentations, and Other Accomplishments:

Zhang, D., and Cowin, S.C. "Load carrying capacity of the pore pressure in a poroelastic beam subject to oscillatory excitation" in "Mechanics of Poroelastic Media." Edited by: Selvaduri, A.P.S. Wolters Kluwer Academic Publishers, Solid Mechanics And Its Application Series, Vol. 35, pp 273-298, 1996.

Autogenic Feedback Training as a Preventive Method for Orthostatic Intolerance

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Project Identification: 199-14-12-14
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/96
Expiration: 10/97
FY 1996 Funding: $150,000
Students Funded Under Research: 6

Responsible NASA Center: ARC

Task Description:

Post-flight orthostatic hypotension has been identified as a serious biomedical problem associated with sustained exposure to micro-G. The general purpose of this research is: (1) to learn the most effective ways of training subjects to produce large, voluntary increases in blood pressure; (2) to determine the effectiveness of this training at counteracting various conditions producing orthostatic hypotension; (3) to understand the cardiovascular, endocrine, and other mechanisms involved; and (4) to determine if certain of the mechanisms discovered to be most prominently involved can be used to produce more effective training.

The first study is entitled: "A comparison of blood pressure feedback training alone vs. multiple response feedback training: Effects on orthostatic intolerance." This study will also determine if there are differences in the training results of subjects with initially high or low orthostatic tolerance. Forty-eight men and women will be assigned to three groups (N=16), matched for orthostatic tolerance (eight low- and eight high-tolerance subjects per group), during initial presyncopal lower body negative pressure (LBNP) tests. Groups are Multiple Response Feedback, Blood Pressure Feedback only, and No Treatment Control.

Current NASA research in this area is directed toward understanding the mechanism by which subjects gain voluntary control. Impedance cardiography techniques have provided the first evidence that subjects can learn to modify such measures as cardiac output, stroke volume, central fluid volume, and vagal tone.

Because AFTE enables self-regulation of autonomic responses, it has other applications in addition to the treatment of motion sickness and space motion sickness. Recent studies have successfully demonstrated the potential of AFTE to improve pilot performance under emergency flying conditions. In each of these applications, the same AFTE methods are used to train control of autonomic responses. The only difference lies in the way in which this control is applied. In motion sickness inducing conditions, the subject learns to maintain physiological responses at his own resting level whereas in blood pressure experiments, the subject learns to increase levels (e.g., heart rate, peripheral resistance) sufficiently to overcome the effects of a

gravitational stimulus (e.g., tilt-table). By including this suite of impedance cardiography measures, it will be possible to gain a better understanding of the mechanisms by which subjects learn voluntary control of these responses. Further, we will be able to identify those physiological characteristics of individuals who are susceptible to both space motion sickness and post-flight orthostatic intolerance.

AFT is a method for training human subjects to voluntarily control several of their own physiological responses within a six-hour instruction program. The primary uses of this treatment are: (1) to facilitate adaptation to environmental stressors; (2) improve operator performance; and (3) correct disturbances in autonomic function. AFT has been tested during shuttle missions as a treatment for space motion sickness, during ground-based tests for terrestrial motion sickness, and in high-performance military aircraft for air-sickness. Additional applications include improved pilot performance during emergency search and rescue conditions, as a countermeasure for orthostatic intolerance in aerospace crews, and as a treatment for clinical patients suffering from hypotension, hypertension, nausea resulting from chemotherapy, and other disorders related to autonomic dysfunction. AFT can also be used to modify central nervous system (CNS) activity in the treatment of neuropathological disorders such as epilepsy, attention deficit disorder, and mild head trauma. Neurofeedback training has been used to alter brain activity resulting in the ability to modify effects of sleep deprivation on cognitive performance, and to facilitate sleep by reducing disturbances in circadian rhythmicity.

Specific examples of application of AFTE benefits for Earth currently being investigated are:

1. Space Act Agreement in progress with University of Tennessee: AFTE as a Potential Treatment for Chronic Intestinal Pseudo-Obstruction Syndrome (autonomic neruopathy, symptoms of vomiting, nausea, and syncope).

2. Space Act Agreement in progress with University of Pennsylvania: AFTE as a Potential Treatment for Meniere's Disease symptoms of nausea and syncope.

3. Interagency Agreement in progress with U.S. Army Tank-automotive and Armament Command: to evaluate the incidence and frequency of motion sickness episodes in the Command and Control vehicle using ambulatory monitor equipment designed to monitoring autonomic function of crew members in space.

FY96 Publications, Presentations, and Other Accomplishments:

Cowings, P.S. and Toscano, W.B. Monitoring and correcting human autonomic function during long-term adaptation to microgravity. The annual meeting of the American Autonomic Society and Clinical Autonomic Research Society, Toronto, October, 1996. Clinical Autonomic Research, 6(5) p305.

Cowings, P.S., Stout, C., Toscano, W.B., Reynoso, S., DeRoshia, C., and Miller, N.E. The effects of promethazine on human performance, mood states and motion sickness tolerance. NASA Tech Brief, 110420, (November, 1996).

Microvascular Changes During Microgravity

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Funding:

Project Identification: 199-08-17-72/P01HL2958712
Solicitation:
Initial Funding Date: 3/95
Expiration: 2/96
FY 1996 Funding: $
Students Funded Under Research: 3

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 objectives and goals of this project remain unchanged from the original application. 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.

Based on our findings in the previous year that suggest that simulated microgravity is associated with suppression of the renin-angiotensin system, we have now focused our attention on how this suppression may impact on microvessel growth and function independent of changes in pressure.

1. Blockade of the renin-angiotensin system eliminates the angiogenic response to exercise. Countermeasures such as isometric exercise and resistance training have proven to be relatively ineffective in preventing cardiovascular deconditioning during space flight. We hypothesized that since the renin-angiotensin system appears to be involved in microvascular growth, under conditions in which the renin-angiotensin system was suppressed, the angiogenic response to exercise training might be eliminated. Studies were performed in male rats at 10 weeks of age. Groups of sedentary rats (caged controls) or rats exercising on a treadmill (Walkers: 20 meters/hr @ 5% grade, Runners: 60 meters/hr @ 5% grade) were studied. Each group consisted of equal numbers (n = 14/group) of rats whose renin-angiotensin had been blocked using the converting enzyme inhibitor Captopril (100mg/Kg/day p.o.) and unblocked normals. As previously described, moderate exercise resulted in a rapid angiogenesis in the microcirculation (25% increase in vessel number). In these studies the rapid response to both moderate and intense exercise was completely eliminated by converting enzyme blockade (1692.8 vessel intersections Runners, 1695.4 Walkers, 1633.5 Sedentary). These results suggest that under conditions in which the RAS is suppressed, one of the beneficial effects of exercise, namely exercise-induced angiogenesis, may be eliminated.

2. Microvascular remodeling in microgravity: forces to be reckoned with. During microgravity there are increases in transmural pressure, alterations in flow patterns and a significant remodeling of the microcirculation. We present evidence to support the hypothesis that both elevations in local pressure and flow and modulation of the renin-angiotensin system are stimuli capable of causing remodeling of the microcirculation. Using animal models in which pressure and ANGII concentrations can be altered independently we assessed the importance of physical vs. hormonal factors in microvascular remodeling. RRM and sham operated rats on low (0.4%) or high (4.0%) salt diets were studied. In one group of the animals regional circulations were protected from elevations in pressure by use of a constricting clip. Other groups received subpressor (5 ng/kg/min) or pressor (10 ng/kg/min) infusions of angiotensin II (ANGII). Chronic measurements of blood pressure, plasma renin activity, and plasma ANGII levels , and acute measurements of microvessel density, vascular reactivity, wall area, and tissue angiotensin converting enzyme activity (ACE) activity were performed. In companion studies, endothelial cells of vascular origin were studied in culture to determine potential shear sensitivity of ACE expression and activity. These studies revealed that the rapid microvascular remodeling can occur in the absence of a pressure rise, requires suppression of the RAS, and is reversible with return to low sodium intake. Changes in ACE expression both *in vitro* and *in vivo* suggest that shear stress sensed by vascular endothelial cells may provide a link between local hemodynamic forces and microvascular remodeling. This hypothesis was further supported by a mathematical analysis of estimated shear stress distributions in RRM rats and during microgravity.

3. Effect of renin-angiotensin suppression on vasodilator responses of skeletal muscle resistance arteries: In previous studies we have shown an interaction between local hemodynamic forces and the renin-angiotensin system. We have also demonstrated that during simulated microgravity a profound and rapid remodeling of the

microcirculation occurs which appears to be mediated, in part by suppression of the renin-angiotensin system. In these studies the RAS was modulated not by microgravity but by altering sodium intake. In this way, the role of the renin- angiotensin system in microvascular function could be evaluated independently of a change in microvascular pressure. Resistance arteries (100-200 μm) were isolated from the gracilis muscle of Sprague Dawley rats following short term (3 days) or chronic (4-8 weeks) exposure to either a high salt (HS) diet (4.0% NaCl) or a low salt (LS) diet (0.4% NaCl). The vessels were cannulated with micropipettes and connected to a reservoir that allowed transmural pressure to be maintained at 100 mmHg while the vessel was perfused and superfused with physiological salt solution (PSS). Short-term and chronic exposure to a HS diet both caused a significant reduction in the vasodilator response to acetylcholine, hypoxia (reduction of PSS PO2 to 35-40 torr), and the stable prostacyclin analog Iloprost compared to LS controls. To determine the role of angiotensin II (ANGII) suppression in contributing to the impaired vasodilator responses in animals on the HS diet, one group of rats was chronically instrumented to monitor blood pressure and received a continuous intravenous infusion of a low dose (5ng/kg/min) of ANGII. Rats were fed a HS diet for 1 week and received the ANGII infusion for three days before the arteries were studied. Vasodilator responses to acetylcholine, hypoxia, and Iloprost in arteries of HS rats receiving the ANGII infusion were restored to levels similar to those of the LS rats, suggesting that ANGII suppression in response to the HS diet contributes to an impaired response of skeletal muscle resistance arteries to vasodilator stimuli. Impairment of dilatory response may well impact on the regulatory ability of the cardiovascular system during prolonged exposure to microgravity when suppression of the RAS occurs. These studies suggest that careful monitoring of the status of the RAS, and knowledge of the effect of sodium intake on RAS suppression during weightlessness may be critical to understanding the impact of microcirculatory dysfunction during weightlessness.

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 influence 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 three 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 three 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 bedrest. 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.

FY96 Publications, Presentations, and Other Accomplishments:

Cress, M.E., Conley, K.E., Balding, S.L., Hansen-Smith, F., and Konczak, J. Functional training: Muscle structure, function, and performance in older women. J. Orthopaedic & Sports Physical Therapy, 24, 4-10 (1996).

Greene, A.S., Rieder, M.J., Munzenmaier, D.H., Cooke, A.R., Hansen-Smith, F.M., and Lombard, J.H. (abstract) Microvascular remodeling in renal hypertension: Forces to be reckonded with. Ann. Biomed. Engin., 24(1), S-34 (1996).

Hansen-Smith, F., Hudlicka,O., and Edington, S. *In vivo* angiogenesis in adult rat skeletal muscle: Early changes in capillary network architencture and ultrastructure. Cell & Tissue Res., 286, 123-136 (1996).

O'Drobinak, D.M. and Greene, A.S. Decreases in steady-state muscle performance and vessel density in reduced renal mass hypertensive rats. Am. J. Physiol., 270 (Heart Circ. Physiol. 39), H661-H667 (1996).

Papanek, P.E., Rieder, M.J., and Greene, A.S. (abstract) Captopril blocks aniogenic response to short-term exercise. Circulation, (in press).

Rieder, M.J., Carmona, R., Krieger, J.E., and Greene, A.S. (abstract) Shear stress suppresses angiotensin converting enzyme activity and promotor expression. FASEB J., 10(3), A275 (1996).

Rieder, M.J., Carmona, R., Krieger, J.E., Pritchard, K.A., Jr. and Greene, A.S. Suppression of angiotensin converting enzyme expression and activity by shear stress. Circ. Res. I, (in press).

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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Project Identification: 199-18-17-12
Solicitation:
Initial Funding Date: 7/94
Expiration: 7/97
FY 1996 Funding: $121,133
Students Funded Under Research: 47
Joint Agency Participation: NIH/National Institute of Mental Health

Task Description:

A two-part experimental protocol was designed to test the resetting and the stability of the photic resetting of the human circadian pacemaker. 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). Part B was designed to test the stability of this response in an environment impoverished of circadian time cues (Hypothesis 2) and the gradual regrowth of circadian amplitude following the bright light stimulus (Hypothesis 3). After an 8-h recovery sleep from CR2, the subjects would undergo a third CR to monitor extended responses to the resetting stimulus. As detailed in the FY95 update, data we have gathered during year 1 of the present task 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, 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-h and a 38% reduction in circadian amplitude, which we therefore plan to use in this protocol. We initially planned to study six subjects in each protocol for a total of twelve subjects. On year 2, a preliminary analysis was done after eight subjects had been completed (four per condition) in order to reevaluate the direction of the task. In addition, in 1993, we proposed to carry out studies on the effect of intermittent light vs continuous light exposure on resetting the human circadian pacemaker. Preliminary data have revealed that 87.7% and 63.1% of the resetting response was preserved even when the bright light stimulus was interrupted with uniformly spaced intervals of complete darkness for 43% and 77% of the time, respectively. New data indicate that the maximal phase advance for a weak type 1 resetting occurs when the stimulus is centered later than the initial phase of administration used in these preliminary studies. Therefore, additional data have been collected by centering the light exposure 3.5-h after the initial fitted temperature minimum.

During year 2 of the current grant period, a total of 8 healthy young men (18-30 years) were randomly assigned to the treatment condition (bright light exposure; 9,500 lux) or the control condition (dim light exposure; 10

lux). The aim of the study was to investigate the stability of the phase shifts produced by a single 8-h pulse of bright light centered 2.5-h after the initial endogenous circadian temperature minimum. After 3 baseline days on their habitual schedule and in ordinary indoor room light (150 lux), subject underwent a 32- to 36-h constant routine procedure to assess their endogenous circadian phase and amplitude. After an 8-h recuperative sleep episode, treatment subjects were exposed to an 8-h bright light stimulus (9,500 lux) centered 2.5-h after their initial endogenous temperature minimum. A second 39-h constant routine was done to quantify the phase resetting of the light stimulus (Hypothesis 1). This was followed by a 10-h recuperative sleep episode. To assess the stability of the resetting by light (Hypothesis 2), a third constant routine of 44-46-h was carried out. Control subjects underwent the same experimental manipulations of their rest/activity cycle but remained in dim light (10 lux) throughout the phase resetting trial. This preliminary analysis revealed a mean -0.44 q 0.33-h and a mean -0.67 q 0.30 phase delay in the group of subjects exposed to bright or to dim light, respectively. Additional -.80 q 0.53-h and +0.40 q 0.44 phase shifts were observed between the second and third constant routine in the treatment and control groups of subjects, respectively. No significant differences were observed between the two groups of subjects. Due to the unexpectedly high variance of response in both groups of subjects, we decided to await the results of the plasma melatonin and plasma cortisol samples to better assess the state of the endogenous circadian pacemaker throughout the study. These experiments indicate the importance of gathering more data on the resetting effects of extended light stimulus. In addition, we undertook additional studies of the effect of intermittent light on resetting the human circadian pacemaker. Sixteen healthy young males aged 18-30 years were exposed to three consecutive daily, 5-h bright light stimuli, centered 3.5-h after the initial fitted temperature minimum in order to induce phase advance shift with the light either maintained continuously or interrupted by 12 uniformly spaced darkness intervals of 19.67 minutes each. Core body temperature was recorded continuously by means of a rectal sensor and 32- to 50-h constant routines were carried out before and after the light stimulus. The light stimulus produced a phase advance of + 4.13 q 0.035 and +1.85 q 0.36-h in the subjects exposed to continuous or intermittent bright light, respectively. These phase shifts were statistically different between the two groups of subjects (p 0.0001) and contrasted with the -1.05 q 0.38-h phase delay observed in a control group of 8 subjects who were exposed to darkness instead of light for 5-h centered 1.5 h after the initial fitted temperature minimum. The phase delay in these control subjects is consistent with the slightly-longer than 24-h period of the endogenous circadian pacemaker. Another group of control subjects, exposed to a 5-h darkness stimulus centered 3.5-h after the initial temperature minimum is planned. These results, as well as those collected during year 1 of the current grant period, are the first to demonstrate that bright light can substantially phase shift the human circadian pacemaker even when the light exposure is interrupted by recurrent 19- to 44-minute intervals of complete darkness.

Previous studies of astronauts have documented the presence of circadian rhythms abnormalities, sleep disturbances, and vigilance impairment in astronauts even during relatively short flights. A misalignment between the endogenous circadian timing system and the sleep-wake cycle, altogether with erratic exposure to light among astronauts, is thought to be primarily involved in physiologic and behavioral maladaptation to space flight. Therefore, development of countermeasures which result in rapid entrainment of the circadian system to their required work schedule is important and would allow crew members to avoid the performance decrements arising from circadian disruption. Indeed, our preliminary studies suggest that, with careful planning, bright light exposure during the pre-launch period could be done much more efficiently. Refinement of this technology and its incorporation into the work environment of the orbiter could be a significant advance in relieving the deleterious consequences of the extended duty hours and shifting work schedules required during this continuous operation. This will require the induction and maintenance of complete physiologic adaptation of the human circadian timing system to the work schedules required during these missions. The results of the experiments conducted during year 1 and year 2 of the current grant period have major implications for understanding the effect of intermittent and/or erratic exposure to light among astronauts during spaceflight. Better understanding of the basic mechanisms underlying this responsiveness to intermittent light is necessary to ensure stable entrainment of the circadian system during space flight. These studies, as well as those planned in year 3, will lead to the refinement of Kronauer's mathematical model of photic resetting of the human circadian clock and the design of new lighting regimens to further adjust crew members to their working environment. During year three of the present grant, we plan to further test the stability of the resetting effect of a single cycle of light exposure in an environment impoverished in light exposure. Analyses of the plasma melatonin and

plasma cortisol collected during our phase-resetting trial using one single 8-h exposure to 9,500 lux centered 2.5-h after the initial fitted temperature minimum are planned during year 3. These analyses will allow us to redirect the task (by modifying the duration and phase of the light stimulus) and to run additional studies. We predict that a properly-timed exposure to a single bright light stimulus prior to lift-off can enable crew members to reduce and/or eliminate the sleep deprivation and consequent fatigue and impaired performance due to misalignment of circadian phase. The present study also has important implications for the treatment of circadian rhythm disorders, since continuous exposure to bright light exposure may not always be achievable in the field. Indeed more than 7 million Americans work at night, either on permanent shifts or on schedules requiring a rotation of day, evening, and night work. These workers forego nocturnal sleep and then attempt to sleep during daytime hours. Yet, complete physiologic adaptation of endogenous circadian rhythms to such inversion of the daily routine usually fails to occur. We conclude that the use of this technology could have a positive effect on the health and productivity of both crew members in space as well as night shift workers here on Earth.

FY96 Publications, Presentations, and Other Accomplishments:

Boivin, D.B., Duffy, J.F., Kronauer, R.E., and Czeisler, C.A. Dose-response relationships for resetting of human circadian clock by light. Nature, 379, 540-542 (1996).

Jewett, M.E., Kronauer, R.E., Rimmer, D.W., Duffy, J.F., Klerman, E.B., and Czeisler, C.A. The human circadian pacemaker is sensitive to light during the subjective day. Abstracts, 5th meeting, Society for Research on Biological Rhythms, 1996:129.

Neural Mechanisms of Adaptation to Altered Gravity

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Funding:

Project Identification: 199-16-12-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 10/95
Expiration: 10/97
FY 1996 Funding: $154,000
Students Funded Under Research: 4

Responsible NASA Center: ARC

Task Description:

This work is designed to determine the neural mechanisms underlying sensory-motor adaptation to altered G so that the process can be facilitated or accelerated and "side effects" seen early in the process (e.g., ataxia, motion sickness, disorientation, perceptual illusions, disequilibrium) can be minimized. In the proposed series of studies, the relationship between changes in sensory-motor function (e.g., control of posture and spatial orientation) during adaptation to altered G, and associated changes in morphology, physiology, and neurochemistry of portions of the sensory-motor control systems (e.g., vestibular system, cerebellum, sensory-motor cortex) during adaptation will be determined. Data will be obtained from rats during readaptation to 1-G following chronic exposure to hyper-G produced by centrifugation. The overall goal of these studies is to provide an understanding of the neural processes underlying sensory-motor adaptation to different gravitational environments. Four specific questions will be addressed in this next funding period: 1) Is sensory-motor adaptation to altered G similar to other forms of sensory-motor adaptation (e.g., altered vision, vestibular compensation) in that active, voluntary movement, and previous experience with the altered condition facilitates the adaptation process? 2) What is the significance to the adaptation process of the increased brainstem and cerebellar levels of thyrotropin-releasing hormone (TRH) and Substance P (SP) found following chronic exposure to 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.

The major effort in this funding period has been on the development of, and data collection for, electrophysiological studies of the otolith-spinal reflex following hyper-G exposure. We have used the otolith-spinal reflex response to sudden free-fall to monitor sensitivity in the otolithic system following chronic

exposures to hyper-G, hypothesizing that the amplitude of the early EMG response would be lower in animals exposed to hyper-G than in animals experiencing only the normal 1-G environment. A free-fall testing apparatus was constructed which provides a sudden, vertical linear accelerative, "free-fall" stimulus to the animal which is instrumented with EMG electrodes in the gastrocnemius muscle of the hindlimb. The EMG response, plus acceleration of the animal, were recorded within 3.5 - 9 hours following chronic (7 - 14 days) exposures to hyper-G produced by centrifugation. Analysis of data showed that hyper-G animals had significantly more low-amplitude responses to the free-fall stimuli (suggesting low sensitivity or gain) than did normal, control animals. Animals exposed only to the rotational component of centrifugation had response amplitudes similar to those seen in the control animals. The results of this study suggest that chronic exposure to increased gravitational conditions leads to a decrease in the sensitivity of the gravity-sensing (otolith organ) portion of the vestibular system. This decreased sensitivity may underlie the deficits in otolith-mediated behaviors seen following chronic hyper-G exposure and may be related to the reduced numbers of synapses found in otolith organs under the same conditions of hypergravity. These results provide important information about modifications to the otolith organ system in altered gravitational environments. (Daunton, et al., 1996; Fox, et al., 1996).

In behavioral studies designed to determine the effects of repeated exposures to altered G on neuromuscular system function it was found that repeated 7-day exposures to hyper-G did not significantly improve vestibular function (Corcoran, et al., 1996), unless animals practiced the particular behavior being assessed under the altered G conditions. This result suggests that repeated exposures to altered G conditions alone do not lead to improvement of performance in altered G (Corcoran et al., 1996).

Further data analysis was done on the effects of hyper-G on neuropeptides in the brains of rats exposed to hyper-G for 14 days. Levels of beta-endorphin, CCK, met-enkephalin, somatostatin, Substance P, and TRH were assessed in the brainstem, cerebellum, hypothalamus, striatum, hippocampus, and cerebral cortex. In animals exposed to 2-G, only TRH levels in the brainstem, cerebellum (increased), and striatum (decreased), plus met-enkephalin levels in the cerebral cortex (decreased) were significantly affected by the altered G exposure. Exposure only to the rotational component of centrifugation, however, appeared to have a different effect on levels of neuropeptides. In rotation control animals, TRH levels in the striatum were decreased, met-enkephalin levels were increased in the cerebellum and decreased in the cerebral cortex, while CCK in the brainstem was increased. Since levels of the stress hormone corticosterone were elevated in the rotation control animals, but not in those exposed to hyper-G, it is likely that the changes in neuropeptide levels seen in the animals exposed only to the rotational component of centrifugation are responses to stress. Immunocytochemical studies are planned to determine specifically where the changes in TRH levels occur, since this neuropeptide is known to play a role in adaptation to other situations in which postural and locomotor control is disrupted, and may provide an approach to facilitate the process of adaptation to altered G (Daunton, et al. in preparation).

Work on the participation of the inhibitory neurotransmitter GABA in the process of neural adaptation to altered G has continued, with results indicating that in areas involved in sending motor control commands (sensorimotor cortex and Purkinje cells in the cerebellum) there is a significant decrease in GABA-immunoreactive cells and terminals. These results suggest that modulation of local-circuit GABA neurons is affected by chronic exposure to altered G and are consistent with our findings of altered control of posture, orientation, and locomotion after the same periods of altered G exposure (D'Amelio, et al., 1996; Polyakov, et al., 1995; 1996).

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.

FY96 Publications, Presentations, and Other Accomplishments:

D'Amelio, F., Fox, R., Wu, L., and Daunton, N. Quantitative changes of GABA-immunoreactive cells in the hindlimb representation of the rat somatosensory cortex after 14-day hindlimb unloading by tail suspension. J. Neurosci. Res., 44, 532-539 (1996).

D'Amelio, F., Fox, R., Wu, L., Daunton, N., and Corcoran, M. Effects of microgravity on muscle and cerebral cortex: A suggested interaction. Advances Space Res. (in press).

Daunton, N. NASA Special Achievement Award (1996).

Daunton, N. Neural and Behavioral Adaptation to Altered G. NASA Space Studies Board, ARC (June, 1996).

Daunton, N. Neural and Behavioral Adaptation to Altered G. NASA Neurolab Project, ARC (September, 1996).

Daunton, N., Fox, R., Corcoran, M., and Wu, L. (abstract) Exposure to hyper-g affects early EMG response to free fall in the rat: Preliminary findings. ASGSB Bulletin, 10, 9 (1996).

Daunton, N.G. "Adaptation of the vestibular system to microgravity" in "Handbook of Physiology: Environmental Physiology, III: The Gravitational Environment, 1: Microgravity." Edited by: Fregly, M.J. and Blatteis, C.M. New York: Oxford University Press, pp 765-784, 1996.

Fox, R., Daunton, N., and Corcoran, M. Study of adaptation to altered gravity through systems analysis of motor control. Advances Space Res. (in press).

Meza, G., Bohne, B., Daunton, N., Fox, R., and Knox, J. "Damage and recovery of otolithic function following streptomycin treatment in the rat" in "New Directions in Vestibular Research." Edited by: Highstein, Cohen, and Buttner-Ennever New York: New York Academy of Sciences, pp 666-669, 1996.

Polyakov, I., D'Amelio, F., Daunton, N., Fox, F., Corcoran, M., and Wu, L. (abstract) GABA immunoreactive cells and terminals decrease in cerebral and cerebellar cortex of rats exposed to hypergravity: Preliminary findings. Neurosci. Abstracts, 22, 1297 (1996).

Wade, C.E., Harper, J., Daunton, N., Corcoran, M., and Morey-Holton, E. Body weight gain during altered gravity: Spaceflight, centrifugation, and return to 1G. J. Grav. Phys., (in press).

Wu, L.-C., D'Amelio, F., Polyakov, I., Daunton, N., and Fox, R. Affordable image analysis system to quantify immunoreactive terminals in the somatosensory cortex. Cell Vision, 3, 249 (1996).

Wu, L.C., D'Amelio, R., Fox, F., Polyakov, I., and Daunton, N. Light microscopic image analysis system to quantify immunoreactive terminal area apposed to nerve cells. J. Neuroscience Methods (in press).

Lower Limb Response to Impact Loads in 1G and Micro-G

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Funding:

Project Identification: 199-26-17-18
Solicitation: 95-OLMSA-01
Initial Funding Date: 2/96
Expiration: 1/99
FY 1996 Funding: $155,914
Students Funded Under Research: 1

Task Description:

Exercise in microgravity is one of the most promising countermeasures to the dual problems of space flight-induced bone loss and muscle atrophy. Although exercise in microgravity has been studied extensively from a metabolic standpoint, little research has focused on the efficacy of different forms of exercise for maintaining musculoskeletal integrity in this unique environment. Exercise protocols thus far have not been effective in preventing muscle atrophy and bone loss during space flight, especially in the lower extremities. In 1-G, however, animal experiments have clearly indicated that (i) certain bone strains and strain rates do stimulate bone deposition, and (ii) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column. Such studies have also indicated that a relatively small number of appropriate loading cycles may lead to bone deposition. This suggests that an optimal exercise regimen might be able to maintain bone and muscle integrity during space flight.

Since there is evidence that the bones and muscles of the lower limbs are particularly affected by space flight, the proposed study will address two major aims: (1) to determine the relationship between (i) externally applied impact loads and rates of loading and (ii) the (global) strains and accelerations in the calcaneus and tibia *in situ* and *in vivo* in 1-G, and (2) to determine the external loads, rates of loading, global strains in the calcaneus, tibial accelerations, and the amount of eccentric and concentric whole-muscle activity during jumping exercises in true and in simulated zero-gravity. Each of these aims will be addressed by well-defined, interrelated experiments. To address the first aim, cadaver experiments will relate the global strains and accelerations in the calcaneus and tibia to each other and to external loads and rates of loading. Subsequent human *in vivo* trials will relate tibial accelerations and global strains in the calcaneus to external loads elicited by jumping exercises. How such loads can be achieved in zero-gravity will be investigated with a simulator that negates the effect of gravity on a subject's limbs. The experiments in the simulator will be validated with KC-135 aircraft experiments in which true zero-gravity is achieved.

The overall goals of this proposal are 1) to demonstrate that jumping exercises may be more effective and efficient than current exercises performed in zero-gravity with respect to maintaining bone density and muscle strength; 2) to validate the zero-gravity simulator as an appropriate substitute for true zero-gravity experiments during development of an optimum exercise regime; and 3) to quantify relationships between external loading

profiles and internal bone strains. This knowledge will not only benefit planners of an in-flight exercise program, but it is also expected that the novel experimental techniques will provide valuable information in the development of exercise-based countermeasures for osteoporosis and muscle atrophy. Moreover, if the zero-gravity simulator is shown to be an appropriate substitute for true zero-gravity experiments, it will provide a much less expensive way to conduct some experiments.

In the first year of the project, the focus has been on (i) obtaining hardware needed for the research (and where necessary, designing and testing new hardware), (ii) installing a zero-gravity simulator that will be used in year 2 for studying possible countermeasures to bone loss in microgravity, and (iii) performing cadaver trials to examine shock transmission through the calcaneus.

Preliminary analyses of cadaveric test data revealed a tensile strain rebound following compression in both the tibia and calcaneus. This observation is reasonable since we are performing an impact test within the linear elastic range of loading of the bone. We are also considering other possible sources for this rebound signal, including oscillation of the extensometer itself. The range of peak loads generated corresponded to 2-10 times body weight and peak tibial and calcaneal strains were in the range reported by investigators who have measured cortical bone strain *in vivo*. Because of the range of peak loads and tibial strains we have measured so far, we believe our test protocol is reasonable and is loading the specimens over a range that has an upper limit close to the maximum load that would be encountered in normal circumstances in 1-G.

Based on the tests to date, we can say that (i) we are able to perform the tests and collect the data we set out to collect; (ii) peak loads are comparable to loads produced by jumping exercises *in vivo* and tibial strain data are comparable to values reported in the literature (there are no available comparative values for the strains observed in the calcaneus); and (iii) preliminary analyses indicate significant relationships between external forces and internal bone strains and strain rates. This means we will likely be able to develop more general relationships for use in prescribing exercise-based countermeasures to lower limb bone loss.

While this research is aimed at developing appropriate countermeasures to prevent bone loss and muscle atrophy in astronauts, it also has applications for humans on Earth. For example, osteoporosis is the degeneration of bone and is a factor in more than 1.5 million fractures per year in the United States. There are many possible mechanisms which cause osteoporosis, one of which is the magnitude of forces applied to the bone. Bone is a living tissue which is constantly breaking down and rebuilding itself. The manner in which it does this is dependent, among many other things, on the loads that it experiences. Activities of daily living, such as walking, are generally associated with forces being applied to the bone which cause the bone to maintain its normal strength. However, when a person is bed-ridden or loses the ability to perform normal activities of daily living, the forces applied to the bones are reduced and thus the strength of the bones is reduced. This results in a danger of bones breaking under circumstances in which they would not normally break, such as tripping or falling.

Zero gravity induced osteoporosis can be examined to investigate the amount of force necessary to maintain normal bone mass. Thus, efforts to determine the stimuli (in terms of magnitude of force, the rate of force application, and the frequency of the force application) necessary to prevent bone loss in astronauts will also provide us with a basic understanding of the factors affecting bone formation/degradation in general and will aid in our understanding of osteoporosis and measures which may be undertaken to prevent or slow down this process. Likewise, an understanding of muscle atrophy in astronauts and appropriate measures to counteract this loss will translate to a greater understanding of muscle atrophy in the general population, such as that associated with disuse or aging.

FY96 Publications, Presentations, and Other Accomplishments:

Davis, B.L., Cavanagh, P.R., Sommer, H.J. III, and Wu, G. Ground reaction forces during locomotion in simulated microgravity. Aviation, Space and Environ. Med., 67, 3, 235-242 (1996).

Acoustic Bone Mass and Trabecular Property Measurements

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Funding:

Project Identification: 199-80-07-04
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $99,996
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 for subsonic and the other for ultrasonic measurements. A series of tests was performed using both the ultrasonic and subsonic techniques.

The ultrasonic tests were aimed at measuring nonlinear parameter of trabecular bones. It was observed experimentally that porous media such as sea sediments, sandstone, soils, have anomalously high value of the nonlinear parameter (102 - 103) as compared with non-porous media (less than 10). We assumed that porous media such as trabecular bone may also be highly nonlinear. A theoretical investigation demonstrated that the nonlinear parameter correlates with porosity of the medium, thus making the nonlinear measurements informative for assessment of trabecular properties. The theoretical approach is based on Biot's semilinear model of liquid-saturated porous media. This model assumes that liquid and solid matter of the porous medium exhibit linear behavior within a wide practical range of stresses. On the other hand, the strain due to the dynamic (acoustic) stresses involves modification of local geometry in the pores. These modifications are essentially nonlinear even for small strains. Such mixed behavior leads to nonlinear stress-strain relations. The derived nonlinear model establishes a correlation between the measurable effective nonlinear parameter and structural parameters of the medium

The experimental investigation has been carried out in the frequency range from 1 to 800 kHz. The major problem in measuring the nonlinear parameter is the nonlinear interference in the electronic equipment. High dissipation of acoustic waves in trabecular bone in the frequency range above 100 kHz also makes it difficult to measure the nonlinear effects. Different approaches to measure the nonlinear parameter were tested. It appeared that the most effective approach is to measure interaction of higher frequency ultrasound with lower frequency vibration. Thus, the nonlinear parameter were measured for three ultrasonic frequencies 26, 37, and 60 kHz modulated by the vibration frequencies 4.6 and 9.8 kHz. *In vitro* determined value of the nonlinear parameter for

bovine bone used in the tests was in the range 80 - 120, which is an order of magnitude higher than in non-porous media (e.g. compact bone and liquid).

While ultrasonic measurements can deliver information about the microstructure of bone, a subsonic technique is intended to provide information regarding the overall mass of bone. The proposed subsonic method employs the measurements of the "rigid body" resonance of a tibia or ulna with the use of an artificial spring with a known stiffness. The spring shifts the resonance into very low (subsonic) frequency range, thus simplifying the biomechanical model and interpretation of the measurements. Computer simulation and experimental tests with a simulated bone and human tibia (*in vivo*) proved the concept of the method. The frequency range was determined in which the effect of damping does not interfere with the measurements. The correlation between the measurements and bone mass was observed.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Donskoy, D.M. and Bang, G. Dynamic shear modulus measurement technique for very soft materials. J. Acous. Soc. America, 99(4), 2536 (1996).

Donskoy, D.M., Khashana, K., and McKee, T.G. Application of Biot's poroelasticity theory for nonlinear acoustics. J. Acous. Soc. America, 99(4), 2487 (1996).

Modulation of Bone Remodeling via Mechanosensitive Channels

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Funding:

Project Identification: 199-40-47-04
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $125,807
Students Funded Under Research: 2

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. We previously found that high magnitudes of chronic, intermittent mechanical strain (CMS) altered the SA-cat channel kinetics. In the original grant, we proposed to examine the effects of different magnitudes and frequencies of CMS on SA-cat channel kinetics, intracellular calcium, interaction with calciotropic hormones and integrin-cytoskeletal interaction. However, recently we have developed a mechanical loading apparatus which can alter the levels of strain and fluid shear independently. Using this apparatus, we have found that physiologic levels of mechanical strain has no effect on osteogenic markers such as osteopontin and prostaglandin synthesis. Instead these markers are enhanced by the fluid shear across the surface of the cells. Therefore, we have altered this grant to include examination of the effects of fluid forces on osteoblast function. In these studies we plan to apply different levels of fluid shear and mechanical strain to osteoblasts and osteoblast-like cells, *in vitro*, and in conjunction with patch clamp analyses and cellular and molecular techniques, examine the role of mechanosensitive channels in: 1) the osteoblastic response to varied magnitudes and frequencies of both strain and fluid shear, and the interaction of these two types of mechanical stimuli; 2) the intracellular calcium response to these mechanical stimuli; 3) the interaction of fluid shear and strain with hormonal stimulation on osteoblastic function; and 4) the relationship of these mechanical stimuli with the extracellular matrix-integrin-cytoskeletal axis. These studies will provide important information on how bone responds to the mechanical environment and perhaps, how the loss of mechanical stimulation, as in weightless conditions, alters calcium balance during extended space flights.

We made three significant observations during this funding period. First, we have found that fluid shear, and not physiological levels of mechanical strain, produce an osteogenic response in cultured osteoblasts. This finding would begin to answer a fundamental question of what type of mechanical stimulus most influences the cellular response to loading. However, this raises another question. Does the type of mechanical strain a cell perceives dictate its response? We have developed a new mechanical loading device which can independently alter fluid shear and strain to initiate these studies. The second significant observation made was the identification of the SA-cat channel in UMR-106.01 cells as an alternatively spliced isoform of the a1 subunit of an L-type voltage-sensitive calcium channel (VSCC). We have found that expression of this isoform is increased with mechanical loading and is dependent on fluid shear and not mechanical strain. Combining the loading studies with the antisense strategy, we will be able to determine the role each isoform of the VSCC plays in mechanotransduction and the hormonal stimulation of osteoblasts. Finally, we found that the extracellular

matrix plays a marked role in determining the functional expression of channels. When cells are grown on glass, the channel isoform responsible for the intracellular calcium transient induced by swelling was different from the isoform mediating this response when cells were grown on tissue culture plastic or type I collagen. These data would suggest that the cellular response to varied stimuli could be dependent on the matrix to which the cell is attached. It is well known that non-collagenous proteins are embedded in the bone matrix, yet their physiologic function has yet to be clarified. Thus we will continue to examine the effects of both mechanical and hormonal stimulation on the phenotypic response of osteoblasts grown on different types of matrix proteins.

The mechanical environment is vital to the function of many physiologic systems, but perhaps none as key as to bone. Removal of mechanical stimulus, as in immobilization or space flight, produces a rapid loss of total body calcium, a decrease in bone matrix proteins and a reduction in bone mass, ultimately producing an osteoporotic condition termed immobilization osteoporosis. Conversely, application of mechanical stimulus to bone increases bone mass and can retard bone loss induced by other pathologies, such as postmenopausal osteoporosis. Illumination of the cellular mechanisms responsible for the transduction of mechanical stimuli into cellular biochemical responses will provide both physiologic and pharmacologic foci to attempt to provide methods to increase bone formation, a critical medical concern to the aging population. Furthermore, with the possibility of conservation of mechanotransduction mechanisms in other systems, these studies could provide valuable insight into medical problems such as hypertension.

FY96 Publications, Presentations, and Other Accomplishments:

Duncan, R.L. "Mechanical stimulation of single cells: Measurement of mechanically sensitive channels" in "Signal Transduction - Single Cell Research." Edited by: Van Duijn, B. and Wiltink, A. Springer-Verlag, Heidelberg-New York, (in press).

Duncan, R.L., Kizer, N., Barry, E.L., Friedman, P.A., and Hruska, K.A. Antisense oligodeoxynucleotide inhibition of a swelling-activated cation channel in osteoblast-like osteosarcoma cells. Proc. Natl. Acad. Sci, USA 93, 1864-9 (1995).

Postural Effects on PTH, Calcium, and Skeletal Dynamics

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Project Identification: 199-26-17-14
Solicitation: 93-OLMSA-07
Initial Funding Date: 7/95
Expiration: 6/97
FY 1996 Funding: $116,040
Students Funded Under Research: 1

Task Description:

Marked bone demineralization and severe hypercalciuria resulting in an increased risk for stone precipitation are two salient features of the weightless state. The mechanisms responsible for these changes are unclear.

Project 1: Effect of postural changes on calcium, PTH, and bone dynamics.

Update: Previous studies unraveled a number of abnormalities in response to the weightless state including disturbances in the amplitude and period of various circadian rhythms. There is an increasing body of evidence supporting an anabolic effect of parathyroid hormone (PTH) circadian rhythms on bone remodeling, either directly through its effects on bone remodeling or indirectly through decreasing urinary calcium excretion. We recently implemented a constant routine protocol, and demonstrated that when subjects are semirecumbent, fed hourly meals, and kept in a state of forced wakefulness, that the amplitude of PTH circadian rhythm is blunted and that the amplitude of urinary calcium excretion is increased. The impact of changes in posture per se on the above mentioned rhythms is, however, unclear. In this project we sampled PTH, ionized calcium, and other serum and urinary indices of mineral and bone metabolism every 20-60 min for 24-36 hours under baseline conditions followed by 40 hours of constant posture (CP) in 10 healthy male subjects. Under CP there was a blunting in the amplitude of PTH circadian rhythm that was accompanied by a marked increase in urinary calcium, sodium, and N-telopeptide excretion. The fact that mean serum Cai levels throughout the CR decreased during the CP suggest that the primary event is not increased bone resorption but a blunting in PTH amplitude, decreasing its anabolic effect, thus resulting in increased bone resorption and enhanced urinary calcium excretion. The above mentioned changes in the indices of calcium and bone metabolism took place very early on during the onset of CP, a protocol partially simulating weightlessness, suggesting that the blunting in PTH amplitude and the catabolic bone remodeling profile occurs very early during the loss of gravitational forces.

Project 2: Effect of postural changes on urinary calcium and sodium handling.

Update: The essential role of the kidney in calcium balance is undisputed and significant alterations in renal hemodynamics and sodium homeostasis have been noted during simulation of the weightless state. Most studies evaluating urinary calcium handling by administering calcium have been limited by the fact that as Ca levels increase PTH levels gradually decrease and by the fact that indices of renal hemodynamics (that could affect calcium handling) were not evaluated. We have developed a calcium handling protocol, monitoring renal blood flow and glomerular filtration rate, under a PTH clamp to evaluate Ca-dependent (PTH independent) renal

calcium handling. Its implementation, in eight subjects studied during the semirecumbant posture revealed that not only urinary calcium but also urinary magnesium and sodium excretion are all tightly modulated by increments in Cai, levels. These observations suggest that the natriuresis documented in the weightless state may be closely modulated by changes in urinary calcium excretion outlined in our first project, thus proposing a novel mechanism that may partially explain the decrease in extracellular volume that takes place with the weightless state. Implementation of the same experiments in subjects in the erect position over the coming year will allow a dissection of the impact of posture on these metabolic changes.

Task Progress and Future Directions:

First Specific Aim: Test the hypothesis that the semi-recumbent posture induces a blunting of PTH circadian rhythm and a negative bone remodeling profile.

Methods: To date, we have studied 10 normal volunteers in the Intensive Physiologic Unit at the Brigham and Women's Hospital. The results presented below reflect data available to date on 4-10 subjects depending on the variable evaluated. The first 36 hours spent under baseline conditions were followed by 28-40 hours of "constant posture" conditions (CP, strict semi-recumbent position at 30E, the schedule for the meals and sleep were identical to the ones for the baseline day). Plasma hormone rhythms were analyzed by first determining the average for each subject within a two-hour bin relative to the subject's regular waketime. The average and standard error across subjects for each two hour bin was then computed. For urinary variables, the value of the urine measure at each time point was divided by the urinary creatinine at that point. All urinary points for all subjects for each variable were pooled and a two-harmonic curve was fit.

Results: The cortisol rhythms demonstrated the expected strong circadian rhythm with a trough around 2 a.m. and a peak around 8 a.m. during both baseline and CP conditions. Cai demonstrated a significant circadian rhythm in 7/10 subjects that varied widely between individuals. A minimum occurred between four and 6 p.m. in both baseline and CP conditions and a maximum around 10 a.m. at the beginning of the CP condition, but not after 24 hours in constant posture. During baseline conditions, PTH levels followed a bimodal diurnal rhythm with an average amplitude of 6 pg/mL. A primary peak (t1max) occurred at 4 a.m. and the secondary one (t2max) at 6 p.m., whereas the primary and secondary nadirs (t1min and t2 min) took place on average at 10 a.m. and 10 p.m., respectively. This rhythm was preserved under CP conditions, albeit with different characteristics, thus confirming its endogenous nature. During the CP, PTH rhythm was unimodal and had a blunted amplitude measured at 5 pg/mL with a broad plateau from 6 p.m. to 6 a.m. The minimum at 10 a.m. was retained. Overall, the rhythms in Cai and PTH were inversely correlated during both baseline (R = -0.6, lag = 0 hour) and CP conditions (R = -0.88, lag = 0 hour).

The amplitude for urinary calcium/creatinine (UCa/Cr), sodium (UNa/Cr) and urinary N-telopeptides (N-Tx, a sensitive marker of bone resorption), circadian fits were all increased by 20-30% during the CP as compared with the previous baseline period. Conversly, urinary phosphate excretion decreased by approximately 30% during the CP protocol. Values of urine N-telopeptide cross-links were highest at 8 a.m., in the first urine sample after awaking during both baseline and CP conditions. The early increase in UCa/Cr and N-Tx/Cr within the first 24 hours of the CP protocol suggest that the increase in bone resorption occurs very early during the decrease in gravitational forces. The increase in urinary calcium and decrease in urinary phosphate excretion suggest that the blunting in PTH amplitude, rather than increasing bone resorption, is the primary event since in the latter instance an increase rather than a decrease in phosphate excretion would be expected. In conclusion, PTH levels exhibit a diurnal rhythm that persists albeit with a blunted amplitude during CP. The clinical relevance of the blunting of the rhythm's amplitude is reflected in the associated changes in the rhythms of biological markers of PTH effect on the kidney, namely UCa/Cr and UPO4/Cr and on bone remodeling, namely N-Tx.

Future directions: Our studies evaluate the effect of short term changes in posture on calcium and PTH dynamics. The impact of prolonged changes in posture on these parameters is unclear and deserves further evaluation. An evaluation of measures aiming at increasing PTH amplitude through PTH administration and/or use of Fosamax may further elucidate the specific role of PTH circadian rhythm on calcium and bone

metabolism and will confirm whether these therapeutic strategies reverse the deleterious metabolic changes that occur in the weightless state, herein simulated by changes in posture.

Second Specific Aim: Test the hypothesis that the semi-recumbent posture disturbs renal calcium handling.

Methods: Eight normal male subjects were evaluated while in balance on a high (200 mEq/d) and then on a low (10 meq/d) sodium (Na) diet with the following clearance protocol of Attie et al. To achieve steady state PTH levels, hPTH (1-34) was infused at 0.2 U/Kg/hr to achieve N-terminal PTH levels at the upper limit of normal and suppressed intact PTH levels of less than 10 pg/ml. PAH and INULIN were administered to evaluate renal blood flow and glomerular filtration rates. Subjects were studied in the semi-recumbent posture. Serum ionized calcium (Cai), iPTH and urine (U) Ca, magnesium (Mg), sodium (Na), and creatinine (Cr) were collected from six 30 min control clearance periods and from six periods while receiving $CaCl_2$ at 75 and then 100 Feq/kg/hr. The curves describing Ca, Mg, and Na excretion as a function of SCai were best fitted by a sigmoidal function defined by a set-point of 1.51 mmol/L, 1.49 mmol/L, and 1.55 mmol/L, respectively. These results document the direct regulation of renal Ca-handling but also Mg and Na by Ca when PTH is clamped.

Future directions: Several lines of evidence suggest that the effect of calcium on the above observed changes in urinary calcium and sodium excretion is mediated through the recently cloned calcium receptor that is known to be located in the thick ascending loop. Calcium receptor antagonists that are currently being developed will further clarify the pivotal role of the calcium receptor on calcium and sodium excretion and may offer a potent therapeutic strategy to prevent the hypercalciuria and natriuresis that result form the weightless state, herein simulated by postural changes.

Our protocols shed important light on the mechanism of immobilization hypercalcemia, hypercalciuria, and bone loss. We demonstrated that the blunting of the amplitude of PTH rhythm in response to the semirecumbent posture is accompanied by hypercalciuria and a catabolic bone remodeling profile. Measures aiming at increasing PTH amplitude through PTH administration and/or use of Fosamax will further elucidate the specific role of PTH circadian rhythm on calcium and bone metabolism and will confirm whether these therapeutic strategies reverse the deleterious metabolic changes that occur in the weightless state, herein simulated by changes in posture.

Similarly, we have developed a protocol that specifically characterizes calcium-dependent calcium and sodium excretion. There was increased renal calcium and sodium excretion in the semirecumbent posture. Our findings suggest that this effect is mediated through the calcium receptor. These studies need to be implemented in the erect state to determine the impact of posture on these changes. The use of medications targeted at the receptor will reverse the negative calcium balance state that may ensue from the weightless state.

The above mentioned therapies are applicable to immobilization hypercalcemia and idiopathic hypercalciuria. Once our models are validated to represent biological changes which take place in space, these therapies could also be used to prevent the bone loss experienced by astronauts in space. Finally, such therapies may also have a significant impact on the development of treatment strategies for osteoporosis, a disease affecting 1/3 of women and a significant number of men by age 90. Osteoporosis results in a staggering cost to the health care system of the United States of America. It is estimated to incur an expenditure of 10 billion dollars annually, a number that is on the rise due to the increasing elderly population.

In summary, our studies will bring a new dimension to our understanding of some of the mechanisms responsible for both space flight induced as well as idiopathic osteoporosis and nephrolithiasis, thus allowing the development of novel countermeasure programs to prevent these processes.

FY96 Publications, Presentations, and Other Accomplishments:

El-Hajj Fuleihan, G., Brown, E.M., Gleason, R., Scott, J., and Alder, G.K. Calcium modulation of adrenocorticotropic hormone levels in women. J. Clin. Endocrinol. Metab., 81, 932-936 (1996).

El-Hajj Fuleihan, G., Moore, F., Jr., Gleason, R., LeBoff, M., Angell, J., and Scott, J. Combined estrogen therapy and surgery in hyperparathyroidism. JBMR, 11(S1) Abstract: T607, (1996).

El-Hajj Fuleihan, G., Scott, J., and Brown, E.M. Extracellular Ca regulated Ca handling. Oral presentation at the Annual Meeting of the American Society of Bone and Mineral Research, Seattle WA. JBMR; 11(S1): Abstract 104. 1996.

Porter, L., Colnlin, P., Scott, J., Brown, E.M., and El-Hajj Fuleihan, G. Calcium regulation of the renin-angiotensin system. Annual Meeting of the Endocrine Society, San Francisco, CA. Abstract P1-783, 1996.

Cardiopulmonary Hemodynamics in Microgravity

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Project Identification: 199-14-17-06
Initial Funding Date: 3/94
FY 1996 Funding: $222,000

Solicitation:
Expiration: 3/97
Students Funded Under Research: 5

Task Description:

Ground-based studies have shown a relationship between central venous pressure and cardiac output. In addition, simulations of microgravity have shown that cardiac output increases initially and then returns to control levels over a two to three hour period, over which time blood pressures are tightly regulated at about one Gz levels. The reduction in cardiac output in simulated gravity has been assumed to be due to a reduction in plasma volume; however, this has not been universally demonstrated. Cardiac output and pulmonary blood flow are equal and the distribution of blood flow in the lung is believed to be gravity-dependent. Based on this discussion, during microgravity, pulmonary blood flow should increase with an increase in capillary recruitment, and thus blood flows to the upper parts of the lung. Under this condition, the lung's distribution would be more homogeneous. Results from recent space flight experiments demonstrated that cardiac output was increased in space. In spite of an absence of an increase in central venous pressure, cardiac output remained elevated for 14 days and cardiogenic oscillations were evident, suggesting that lung blood volume was heterogeneous. Based on these observations, the present study was designed to determine cardiac function and blood volume distribution in the lung using nuclear medicine techniques. The specific hypothesis were that removing gravity would result in: 1) an increase in end-diastolic volume, although the effect on cardiac output would be blunted by a decrease in sympathetic tone and contractility resulting in an increase in end-systolic volume and compliance. If true, this could explain how cardiac output could be elevated in space without an increase in central venous pressure and why cardiac output remained elevated in space (increased sympathetic tone); 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.

The primary purpose of this project was to determine cardiac function and pulmonary blood flow and the distribution of pulmonary blood flow in simulated microgravity and increased gravity. During the first period of this grant we determined these parameters during head-down-tilt and confirmed our hypothesis. Specifically that cardiac output was increased due to an increase in end-diastolic volume, but this was paralleled by an increase in end-systolic volume and decreased heart rate. In addition to cardiac "compliance" was significantly increased, thus suggesting that the increased cardiac output could have occurred without an associated increase in

pressure. Pulmonary blood flow was increased during head-down-tilt experiments; however, the distribution of lung blood volume remained heterogeneous. During this period of the grant we have measured cardiac function and pulmonary blood volume and flow during graded water immersion and during a period of six hours of water immersion. Graded water immersion resulted in a progressive increase in cardiac output, which was due primarily to an increased end-diastolic volume, as there was an increase in end-systolic volume and decreased heart rate. There was no decrease in cardiac compliance in these experiments. The pulmonary blood flow increased progressively with greater depth of water immersion and the pulmonary blood volume distribution became more homogeneous. This points out a difference between the data collected during head-down-tilt and water immersion, both simulation of microgravity. Surprisingly, the cardiac output and blood volume distribution remained unchanged for six hours of water immersion, in contrast to the data previously reported suggesting that cardiac output rose and the fell over two hours of water immersion. During these experiments we also measured diffusing capacity using a CO method. These data suggest that the increased pulmonary blood flow and volume were accommodated by a increase in capillary blood volume (engorgement) and not by increased capillary recruitment. These data appear to establish the importance of sympathetic tone in the pulmonary as well as systemic circulation as well as in maintaining a high cardiac output in six hours immersion. These data would appear to be consistent with the data observed in space.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Shykoff, B.E., Farhi, L.E., Olszowka, A.J., Pendergast, D.R., Rokitka, M.A., Eisenhardt, C.G., and Morin, R.A. Cardiovascular response to submaximal exercise in sustained microgravity. J. Appl. Physiol., 81(1), 26-32 (1996).

Magnetic Resonance Imaging in Assessing Forearm Muscle Fatigue after EVA-Related Tasks

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Funding:

Project Identification: 199-06-11-56
Initial Funding Date: 12/95
FY 1996 Funding: $7,050

Solicitation: 95-OLMSA-01
Expiration: 12/96
Students Funded Under Research:

Responsible NASA Center: JSC

Task Description:

Forearm fatigue is one of the limiting factors of longevity during extravehicular activity (EVA). Quantification of forearm muscle fatigue is necessary to guide design of extravehicular mobility unit (EMU) gloves, EVA tools, and exercise protocols for training EVA crew members. Magnetic resonance imaging (MRI), when enhanced by previous exercise of the muscle under study, can differentiate muscle goups from each other and from adjacent fat and connective tissue. Using MRI, specific muscle groups most used during EVA related hand tasks, and therfore most essential for the performance of EVA, can be identified. Also, magnetic resonance spectroscopy (MRS) can be used as a non-invasive means of determining adenosine diphosphate concentrations in the forearm musculature. We hypothesize that MRI T2 and MRS signal intensities can be used as objective, reliable measures of forearm muscle use.

Three preliminary findings are described that satisfy in part the specific aims of the work as outlined below in the original proposal. These findings also suggest some changes in our approach to finding an objective measure of muscle fatigue.

I. Identification of forearm muscle groups using both high resolution proton density and T2 relaxation time images.

A high resolution scan, and several "snapshots" (T2-weighted scans) of the dominant forearms in six individuals was performed both before and after the exercise protocol described in the original proposal. To summarize, the protocol consisted of four EVA-related hand tasks in a glovebox vacuum chamber using the space shuttle extravehicular mobility unit (EMU) gloves. After a baseline series of scans, four repetitions of this protocol were performed (standard submaximal exercise) before a repeat series of scans. The subject then continued to perform the hand-task protocol to exhaustion, defined as the point at which the individual could no longer attain a maximum voluntary grip contraction force of 70% of baseline, after which a final series of scans were obtained.

An increase in the signal intensity of the T2 weighted scans in certain muscle groups was noted in five of the six subjects post-exercise. The central groups, namely the *pronator quadratus* and the flexor *digitorum profundus*, show a strong signal intensity increase. Edema can be noted as well in the post-exercise scans in these muscle groups. In all but one subject, these two muscle groups showed the greatest signal increase. This is not too surprising, since hand pronation and finger flexion comprise the majority of hand motions needed to complete the assigned tasks in this protocol. These muscle groups showed the greatest amount of increase in T2 weighted signal intensity in five of the six subjects. As already mentioned, one subject did not show any increase in signal intensity despite completion of the hand protocol to exhaustion. While the reasons for this are as yet unclear there are several possible explanations, including hydration status and predominant muscle fiber type in this individual.

II. Proton density as a quantitative measure of muscle use.

The intra-individual value for proton density in the high-resolution scans showed fairly wide variability for two measurements made over one month. This is most likely due to bias in tuning of the radio-frequency coil that is performed before each scan, but could also be attributed to subject hydration status or degree of prior forearm activity. While these variables were addressed in questionnaires given to each subject, no consistent confounding variable, other than the RF tuning, was identified. So, while useful in identifying muscle groups most active in these hand tasks, it appears that the proton density alone is not reliable as a quantitative measure of muscle use.

III. T2 relaxation time as a quantitative measure of muscle use.

The T2 relaxation time changes in the *pronator quadratus* and the flexor *digitorum profundus* were also determined in each of the six subjects. The same protocol mentioned above was used and the study repeated one month later. Preliminary results suggest that the post-exercise change in2 relaxation time as compared to baseline shows little repeat measures variability.

The results of this investigation will provide new information which may be important in a number of situations including physicals assessments of occupational tasks of a repetitive nature which are know to result in muscle fatigue and injury (i.e. keyboard input, assembly line work, sports activities). Physical therapy and occupational rehabilitation of these types of repetitive use injuries could be mitigated by knowledge and techniques validated in this study. Additionally, information gained by study of forearm muscle use in repetitive activities could be used in the design of human-machine interfaces in order to attenuate muscle fatigue and injury potential.

Limb Muscle Function with Unloading and Countermeasures

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Funding:

Project Identification: 199-26-17-08
Initial Funding Date: 2/95
FY 1996 Funding: $137,865

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 7

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 continued work evaluating various exercise countermeasures, and the mechanisms responsible for the HU-induced increase in maximal shortening velocity (Vo) and reduced fiber tension. In the following paragraphs, I will briefly summarize our progress on each of these studies.

1. Countermeasure Studies. In the past year, we have continued our work evaluating the effects of high resistive exercise (ladder climbing) as a countermeasure to HU-induced changes in cell function. Additionally, we have begun studies testing a second high resistive exercise system that involves leg press weight lifting, and we developed another system that will allow us to test the effectiveness of isometric exercise. A. Ladder Climbing. In comparison to the intermittent standing (reviewed FY95 Task Progress), ladder climbing exercise was more effective in attenuating the HU-induced loss of absolute force, normalized force, and stiffness but less effective in restoring the type I fiber maximal shortening velocity (Vo) to the control level. The fact that the fiber Vo remained elevated following ladder climbing was beneficial to the fiber as it allowed peak power to return to near normal despite less than complete recovery in peak force. HU has been shown to shift the pCa-force curve to the right, such that, higher free Ca^{2+} is required for a given percentage of peak force. For example, the Ca^{2+} required for 50% peak force increased from 0.593 ± 0.038 to 0.761 ± 0.033 mM following 14 days of HU. The ladder climbing countermeasure completely prevented any change in pCa-force relationship. B. Weight Lifting. Weight training improved the peak force and power of the fast fibers of the gastrocnemius, but actually induced a reduced force and power in the slow type I fibers of the soleus. One possibility is that the slow fibers were actually damaged during the return from full ankle extension as the animal lowered the weight.

During this phase of the lift the soleus muscle undergoes an eccentric contraction, and the stretch might have torn the atrophied slow fibers. We are in the process of conducting additional studies to confirm this observation. If confirmed, we will conduct EM studies to assess if physical damage actually occurred. In a second experiment, we will weight train the rats but eliminate the eccentric component by removing the weight once full knee and ankle extension is obtained. The question of whether or not heavy resistive isotonic weight training damages slow type I fibers is extremely important, and this issue must be resolved before the optimal exercise countermeasure can be developed.

2. Studies designed to elucidate the mechanisms of the HU induced functional changes. Our hypothesis is that the HU induced increase in type I fiber Vo and some of the decline in peak tension can be explained by an increased myofilament lattice spacing that results from the selective loss of contractile proteins. If our theory is correct, we should be able to shrink the filament spacing by exposing the fibers from the HU animal to various concentrations of dextran, and Vo should decline to the control level at a filament spacing equal to control fibers. With zero dextran, the mean Vo of the type I fibers from the soleus of the HU animals was significantly higher than the control (1.7 vs 1.25 FL/s). At a 5% dextran concentration, the Vo of the HU group was identical to that observed for the control group in zero dextran. Quantitatively, the filament spacing decreased by 20% in 5% dextran. Although we have not yet completed the quantitation, this value appears to be similar to the spacing observed in control fibers in zero dextran.

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

FY96 Publications, Presentations, and Other Accomplishments:

Widrick, J.J., Bangart, J.J., Karhanek, M., and Fitts, R. H. Soleus fiber force and maximal shortening velocity after non-weight bearing with intermittent activity. J. Appl. Physiol., 80, 981-987 (1996).

Effect of Bed Rest on Simulated Shuttle Emergency Egress

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Project Identification: 199-14-11-22
Solicitation: 95-OLMSA-01
Initial Funding Date: 10/96
Expiration: 10/97
FY 1996 Funding: $60,000
Students Funded Under Research: 1

Responsible NASA Center: JSC

Task Description:

Astronauts have never been required to perform an actual emergency egress from the space shuttle. A MODE 5 (unassisted) egress would occur after a landing where the usual ground support crew cannot assist the astronauts in egressing the vehicle. In the ground-based part of this study, human test subjects will be trained in performing the egress procedures. In preliminary data, even without space flight, many persons cannot complete the emergency egress scenario with actual flight hardware. The purpose of this study is to evaluate responses during egress simulation to ascertain whether failure to complete the egress scenario is due to: 1) build-up of CO_2 in the non-conformal helmet; 2) leg fatigue and ischemia associated with wearing an inflated g-suit while walking; or 3) overheating. In the flight portion of this study, crew members will be asked to walk on a treadmill immediately after shuttle landing to simulate the egress scenario and confirm the ground-based results.

Ground-Based Studies:

To assess the effect of G-suit inflation on egress fatigue, twelve subjects have been tested while performing the emergency egress scenario at three different G-suit inflations: 0.0 psi, 0.5 psi, and 1.5 psi. With the G-suit fully inflated at 1.5 psi, only four of the twelve subjects could complete the five minute egress scenario. At 0.5 psi, 10 out of 12 could complete the test. With no suit inflation all subjects successfully completed the test. Oxygen consumption during the walking test was increased significantly by 20-30% with G-suit inflation and inspired CO_2 levels were elevated to approximately 5%. Reasons for subject termination varied among subjects, with most reporting leg fatigue and/or shortness of breath.

To evaluate the G-suit effect, the 12 subjects will walk at one more G-suit inflation pressure, 1.0 psi. To assess the effect of CO_2 build-up in the helmet, subjects will be asked to repeat the tests with the visor of the helmet open. To assess the effect of hyperthermia, body temperature measurements will be taken.

Flight Study:

Six astronauts have completed the egress simulation immediately after shuttle flights. Astronauts are allowed to self-select the G-suit inflation based on their individual orthostatic symptoms. Four of of six crew members so

far have been able to complete the five minute egress protocol. Carbon dioxide in the helmet is elevated to approximately 4 to 5.5% at termination of the walk and crew members report symptoms of fatigue similar to the ground-based studies.

This is an operationally-oriented study to assess the feasiblity of successful emergency egress. The results of this study will directly benefit crew members by identifying the limiting factors to successful egress (leg fatigue, CO_2 build-up in helmet, overheating) and by offering recommendations to address such limitations (altered helmet or G-suit design, recommended G-suit inflation for egress, leg strength training, additional pre-cooling).

Earth-based benefits from this study might relate to applications for workers who must wear protective clothing (e.g., nuclear plant workers), protective breathing systems (e.g., firefighters), or anti-G garments (e.g., high performance aircraft pilots).

Effect of Microgravity on Vascular Cell Function

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-08-17-72/P01HL2958212
Solicitation:

Initial Funding Date: 9/94
Expiration: 8/97

FY 1996 Funding: $
Students Funded Under Research: 3

Joint Agency Participation: NIH/National Heart Lung and Blood Institute

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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: 1) Determine the effects of simulated microgravity on vascular cell pro-oxidant activity. We will measure the effect of simulated microgravity on oxidation of LDL by activated monocytic U937 cells. We will also measure the cellular release of factors involved in oxidation, namely, superoxide and ceruloplasmin; and 2) Determine the effect of simulated microgravity on endothelial cell motility and its regulatory signaling pathways. We will evaluate the effect of microgravity on wound-induced aortic EC movement and its regulatory signal transduction pathways. We will focus on basic fibroblast growth factor (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.

We have assembled a simple system to simulate microgravity using slow rotation to "neutralize" the gravity vector. Adherent bovine aortic EC are grown to confluence in shallow tissue culture wells. The wells are filled to the rim with medium to minimize mixing during rotation, and tightly sealed. In the "simulated microgravity" (SM) treatment, the culture dish is rotated about a horizontal axis; the time-averaged gravity vector, from the perspective of the cells, is zero. A stationary control is used for comparison to usual culture conditions; the gravity vector is constant at 1-G and directed from the luminal aspect to the basal aspect. A second control consists of "upside-down" cells; the gravity vector is constant, directed from the basal aspect to the luminal aspect. This control group is used to examine if altered responses in the SM group are simply due to the time that the cells spent upside rather than to neutralization of the gravity vector. A "Z-axis rotation" control is used to determine whether changes found in the SM group are due to centrifugal force or mixing; the gravity vector is constant in this case and directed parallel to the cell length.

During the last year we have studied whether there are specific genes that are transcriptionally regulated by SM. The differential display procedure was used to determine differences between EC exposed to SM and Z-axis rotation control cells. This reverse-transcriptase (RT)-PCR-based method amplifies mRNA molecules using 24 combinations of eight pseudo-random 5' primers and three anchored oligo-dT 3' primers. In theory, the method has the resolution and sensitivity to amplify the entire mRNA population expressed in cultured cells or tissues, and in practice has been used successfully to detect and clone genes that are differentially expressed under multiple conditions. Of the 5,000-10,000 distinct RT-PCR products detected in the differential display analysis only one product reproducibly showed differential expression; a band of approximately 560 nucleotides was present in Z-axis rotation control cells but was almost completely absent in the cells exposed to SM.RNA samples from three separate experiments (each done at least in duplicate) gave nearly identical results. The band was cut from the gel, re-amplified by PCR using the same primers, ligated with TA cloning vector, and transformed into competent *E. coli* JM109. From 12 clones picked, eight had correctly sized inserts.

The differential expression was confirmed by RT-PCR using distinct internal primers, and by Northern blot analysis using the amplified PCR product from the differential display as a random primer-labeled cDNA probe. The mRNA product was about 2.5 kb, and exposure of cells to SM decreased the steady-state level of expression is to less than half of the control level. The signal strength was very low suggesting that it may not be a very abundant mRNA. We are currently developing an RNAase protection assay to improve the sensitivity of detection of this transcript. DNA sequence analysis of the 558 nucleotide product showed a poly-adenylation consensus sequence immediately before the poly-A tail, consistent with the cloned product encoding an mRNA. The transcript is AU-rich and contains two AUUUA sequences, a motif that bestows mRNA instability and is found in the 3'-untranslated region (UTR) of multiple important regulatory molecules. An extended open reading frame is not present and it is thus likely that the region encodes a 3'-UTR of the mRNA. By comparison to "non-redundant" databases (including EST's) using the Blast program, the sequence lacks significant homology to all known sequences and may thus be novel. Alternatively, the sequence may encode a bovine homologue of a known mRNA in another species for which the sequence in the 3'-UTR is either not known or lacks homology to the bovine sequence.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Ehrenwald, E. and Fox, P.L. Role of endogenous ceruloplasmin in LDL oxidation by human U937 monocytic cells. J. Clin. Invest., 97, 884-890 (1996).

Kaufman, B.R., Madura, J.A., Margolin, D.A., DeLuca, D.J., Fox, P.L., and Graham, L.M. Regional differences in platelet-derived growth factor production by the canine aorta. J. Vasc. Res., 33, 53-61 (1996).

Mukhopadhyay, C.K., Ehrenwald, E., and Fox, P.L. Ceruloplasmin enhances smooth muscle cell- and endothelial cell-mediated low density lipoprotein oxidation by a superoxide-dependent mechanism. J. Biol. Chem., 271, 14773-14778 (1996).

Murugesan, G. and Fox, P.L. Role of lysophosphatidylcholine in the inhibition of endothelial cell motility by oxidized low density lipoprotein. J. Clin. Invest., 97, 2736-44 (1996).

Pitsch, R.J., Goodman, G.R., Minion, D.J., Madura, J.A., Fox, P.L., and Graham, L.M. Inhibition of smooth muscle cell proliferation and migration *in vitro* by antisense oligonucleotide to c-myb. J. Vasc. Surg., 23, 783-791 (1996).

Effects of Artificial Gravity: Central Nervous System Neurochemical Studies

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Funding:

Project Identification: 199-16-17-14
Initial Funding Date: 5/95
FY 1996 Funding: $ 100,000

Solicitation:
Expiration: 5/96
Students Funded Under Research: 9

Task Description:

The objective of this project is to assess neurochemical and morphological changes in muscle receptors and the central nervous system of rats subjected to hypergravity (2X Earth gravity, or 2G) produced by centrifugation. The underlying hypothesis of the project is that alterations of normal gravity alter afferent (sensory) information sent to the central nervous system by muscle receptors. Those changes, in turn, will affect the chemical activity 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 of the somatosensory cortex).

Rats were be subjected to hyper-G for up to 14 days, after which they were euthanized or anesthetized and then fixed by perfusion. 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., GABA) with the light microscope were applied. The principal structures 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 neurotransmitter systems related to these changes, we studied g-aminobutyric acid (GABA) immunoreactivity in rats after chronic exposure to 3-G for 14 days. Immunoreactivity of GABA and was studied in areas of the brain and spinal cord that are involved in motor control: cerebellum (anterior vermis, flocculus), lateral vestibular nucleus, somatosensory cortex (hindlimb representation), and spinal cord (dorsal horn).

Immunoreactivity of GABAergic cells was reduced in the hindlimb representation of the somatosensory cortex in layers Va and Vb of the somatosensory cortex. GABA-containing terminals also were reduced in the same layers, particularly those terminals surrounding the soma and apical dendrites of pyramidal cells in layer Vb. On the basis of previous morphological and behavioral studies of the neuromuscular system of animals subjected to hypergravity and hindlimb suspension, it was suggested that chronic exposure to altered gravity changes afferent signalling and feedback information from intramuscular receptors to the cerebral cortex due to modifications in the reflex organization of hindlimb muscle groups. We propose that the reduction in immunoreactivity of local circuit GABAergic neurons and terminals is an expression of changes in their modulatory activity to compensate for the alterations in afferent information.

Additionally, we developed a desktop computer-based method for quantitative assessment of the area occupied by immunoreactive terminals in close apposition to nerve cells in relation to the perimeter of the cell body (Wu *et al.*, 1997, *Journal of Neuroscience Research*). The method uses Fast Fourier Transformation (FFT) on images from 40 μm-thick coronal sections stained for immunocytochemistry. Sections were visualized using a Leitz Diaplan light microscope and were captured into a Macintosh computer using a Sierra Scientific CCD camera. Processing images with the FFT and then combining original and processed images is used to enhance the terminals. A binary image is then used to perform a thresholding operation to produce an image with terminal areas appearing black on a white background. This methodology provides an objective means for measuring terminal area in which the difficulties of labeling intensity, size, shape, and numerical density of terminals are avoided.

The central objective of this research is to expand understanding of how gravity affects neuromuscular systems that control posture and gait. The project uses an approach of integrated study in which molecular changes in the neuromuscular system are related to the development of effective motor control. The research will characterize neurochemical changes that occur in sensory and motor systems and relate those changes to motor behavior as animals adapt to altered gravity. Thus, this research will identify changes in central and peripheral neuromuscular mechanisms as motor control is reestablished after disruption by exposure to hypergravity. Improved understanding of the relationship of mechanisms of "plasticity" in the neuromuscular system to motor control will suggest mechanisms that could contribute to alterations in motor control during and following space flight. Findings from this research also may have clinical applications. Motor control is disrupted by miscellaneous injuries (e.g., spinal trauma, blunt head injury, stroke, damage to the vestibular system) and disease states (e.g., multiple sclerosis, ALS) that affect various components of the neuromuscular system. Findings from this integrated approach to studying molecular and functional alterations in the neuromuscular system will suggest various neuromuscular structures (e.g., motor neurons, cortex, muscle receptors) and neurotransmitters (e.g., GABA) that may contribute to the development of effective motor control as the neuromuscular system reacts to injury or disease.

FY96 Publications, Presentations, and Other Accomplishments:

Corcoran, M., Daunton, N., Fox, R., Welch, R., and Wu, L. Effects of repeated chronic exposures to 2G on disruption of air righting reflex in the rat. ASGSB Bull, 10, 36 (1996).

D'Amelio, R., Fox, R.A., Wu, L-C., Daunton, N.G., and Corcoran, M.L. Effects of microgravity on muscle and cerebral cortex: A suggested interaction. Adv. in Space Res., (in press).

D'Amelio, R., Fox, R., Wu, L-C., Daunton, N. Quantitative changes of GABA-immunoreactivity in the hindlimb representation of the rat somatosensory cortex after 14-day handlimb unloading by tail suspension. J. Neurosci. Res., 44, 532-539 (1996).

Daunton, N., Fox, R., Corcoran, M., and Wu, L. Exposure to hyper-G affects early EMG response to free-fall in the rat: Preliminary findings. ASGSB Bull., 10, 36 (1996).

Meza, G., Bohne, B., Daunton, N., Fox, R., and Knox, J. "Recovery of otolithic function following streptomycin treatment in the rat" in "New Directions in Vestibular Research." Edited by: Highstein, S.M., Cohen, B., and Bytner-Ennever, J.A. New York Academy of Sciences: New York, pp 666-669, 1996.

Polykov, I., D'Amelio, F., Daunton, N., Fox, R., Corcoran, M., and Wu, L. GABA immunoreactive cells and terminals decrease in cerebral and cerebellar cortex of rats exposed to hypergravity: Preliminary finding. Neurosci. Abstracts, 22, 1297 (1996).

Wu, L.C., D'Amelio, F., Rox, R.A., Polyakov, I., and Daunton, N.G. Light microscopic image analysis system to quantify immunoreactive terminal area apposed to nerve cells. J. Neurosci. Methods, (in press).

Wu, L., D'Amelio, F., Polyakov, I., Daunton, N.G., and Fox, R.A. Affordable image analysis system to quantify immunoreactive terminals in the somatosensory cortex. Fourth International Conference and Workshop on Molecular Morphology, Montreal, Canada, June 3-6, 1996. Cell Vision 4, 78.

Circadian Rhythms in Rhesus: Gravity, Light & Gender

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Co-Investigators:

No Co-Is Assigned to this Task

Funding:

Project Identification: 199-18-17-18
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $156,429
Students Funded Under Research: 6

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, heart rate, drinking, and various performance parameters in male and female rhesus monkeys. Temperature and heart rate are monitored by telemetry and drinking by an electronic circuit. Short-term memory, hand-eye coordination, and other performance parameters are assessed using the psychomotor test system (PTS), developed at the University of Georgia Language Research Center. The PTS consists of a succession of 16 different video-based tasks. Successful completion of a task is rewarded with a pelletized diet. All 16 of our test subjects (8 male and 8 female) are fully trained in all the PTS tasks and are able to obtain all of their food through this system. We have found that the amount of time needed for an animal to acquire the skills it needs to be proficient with the PTS varies between individuals, with some subjects completing training in as little as 5 months, while others require up to 10 months of practice.

During training, the animals are individually housed in standard primate cages in an environmentally controlled room at the California Regional Primate Research Center (CRPRC). The cages are arranged to provide all animals with vidual and auditory contact with conspecifics. Entry into the room is strictly controlled. Daily health checks are performed and the results reported to the CRPRC veterinary staff. Food intake is monitored and the pelletized diet is supplemented with standard monkey chow, if necessary, and the animals are weighed biweekly. The PTS stations used for training are stand-alone systems that are contained on individual carts that can be 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. These facilities were also used

to perform a preliminary study examining the responses of male rhesus monkeys to a shift in the light-dark schedule and to constant lighting conditions.

Our 2-G studies will be performed using a six-meter diameter centrifuge at the Chronic Acceleration Research Unit (CARU). During FY96 we developed and refined a module that will allow us to record PTS, video, temperature, heart rate, activity, and drinking continuously, as well as to collect urine, from rhesus individually housed on this centrifuge. A standard vivarium cage fits within the module and houses the animal. The orientation of the PTS is the same as it is at the CRPRC. Each subject has a PTS computer mounted on the center platform of the centrifuge. Using biotelemetry to record body temperature and heart rate allows us to perform our studies on unrestrained animals. However, we had to perform a significant amount of development to design and produce an antenna that would work within the metal caging environment. The antenna design had to capture the signal from the biotelemetry transmitter, ignore background noise, fit within the cage, and not interfere with the moving elements of the cage. The current, successful design utilizes dipole antennae contained within PVC piping. As an additional benefit, an antenna is placed so that the animal is able to use it as a perch, providing an additional source of environmental enrichment.

We have written programs that will allow us to analyze the behavioral and physiological data using our in-house circadian analysis programs. These programs group the PTS data in 10 minute bins and examine number of trials, percent correct, and average response times. The output of these programs will be compatible with the lab's rhythm analysis program so that rhythms of psychomotor and memory-based task performance, heart rate and body temperature 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 female astronauts.

Intercompartmental Fluid Shifts in Response to Postural and Gravitational Forces

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-14-17-03
Initial Funding Date: 10/93
FY 1996 Funding: $80,000

Solicitation:
Expiration: 10/96
Students Funded Under Research: 0

Task Description:

No additional information was supplied by the principal investigator.

Neurocognitive Function Test for Space Flight Crew Members

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Funding:

Project Identification: 199-16-17-15
Initial Funding Date: 6/96
FY 1996 Funding: $199,242

Solicitation: 95-OLMSA-01
Expiration: 5/99
Students Funded Under Research:

Task Description:

We propose to develop an efficient, sensitive, reliable and cost-effective means for assessing changes over time in individual crew members of several fundamental higher cognitive brain functions. The Neurocognitive Function Test (NFT) would be useful for studying changes in these functions during long duration space missions, and would also have dual-use application in ground-based studies of operational fatigue, biological rhythms, medications, or environmental stressors. Unique to the NFT will be the ability to quickly measure how multivariate combinations of behavioral, physiologic, and neuro-physiologic indices of attentional and working memory functions and fine perceptuomotor control change in an individual crew member over time. The basic research to be performed will include pilot studies to determine stability and retest reliability of behavioral, physiologic and neurophysiologic measures, followed by a laboratory experiment to determine the test's sensitivity to operational fatigue and other sources of impairment, and its ability to validly predict the potential for compromised performance. As a final component of the project, we will perform a small study in which the NFT is used to measure operational fatigue in a more realistic context. This latter component of the project will be completed in collaboration with Dr. Mark Rosekind of the NASA Ames Fatigue Countermeasures Program. In addition to research applications, the NFT could ultimately prove to be a sensitive test of work readiness in a wide range of military and civilian work environments.

This project started in the last half of FY96. Progress during that period was limited to planning and preparation for initiating an experimental study in FY97.

In addition to its use in NASA research studies on the effects of long duration space flight on higher brain function, the potential benefits of the Neurocognitive Function Test could form the basis of a dual use technology for assessing "Readiness-To-Perform" in military and civilian work environments. That is, the Neurocognitive Function Test could help reduce the incidence of tragic accidents related to human error. It could serve to detect impaired attention and alertness (resulting from fatigue, hangover, illness, or other debilitating conditions) in personnel critical for chemical and power plant operation, crisis management, aviation, emergency medicine, and military combat. There is a great societal need and a viable market for such a device.

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 1996 Funding: $146,212
Students Funded Under Research: 4

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 5th through 17th months of work on this grant, we made progress in identifying relevant components of the extracellular matrix (ECM) and integrins that mediate the differentiation and survival of cultured osteoblasts. We used the primary fetal rat osteoblast culture system to study ECM/cell interactions and demonstrated that cellular interactions with fibronectin, a component of the ECM produced by osteoblasts, are required for the progressive differentiation of osteoblasts. Furthermore, we have identified specific integrins expressed in both intact calvarial tissue and by cultured osteoblasts, and identified the subset of fibronectin integrin receptors that are necessary for osteoblast differentiation. Whereas immature osteoblasts are dependent on fibronectin for differentiation, we have recently found that mature osteoblasts become dependent on fibronectin for survival. We are now performing additional experiments to identify other important ECM components and integrins, as well as to determine whether mechanical strain of cultured cells alters the synthesis and distribution of fibronectin or its receptors. We have also initiated animal studies to determine the effects of hindlimb unloading and reloading on the expression of specific ECM and integrin components in the skeleton of the growing rat.

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.

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HeartRate Dynamics During Microgravity Exposure: Data Analysis

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Funding:

Project Identification: 199-14-17-19
Solicitation: 95-OLMSA-01
Initial Funding Date: 6/96
Expiration: 6/97
FY 1996 Funding: $91,260
Students Funded Under Research:

Task Description:

NASA has prioritized the development of new, efficient and inexpensive ways to archive, disseminate and analyze the vast amounts of physiological data obtained during space flight and also during ground-based simulations of microgravity exposure. NASA has also given priority to the investigation of two problems encountered in space flight, both of which influence astronaut behavior and performance: 1) space motion sickness (SMS); and 2) cardiovascular deconditioning, especially during long-term missions. We propose to use spectral and nonlinear analyses of heart rate data as quantitative methods for detecting the presence of these problems, to evaluate potential countermeasures against them, and to study their physiologic mechanisms.

Our objectives are intended to extend our results developed during our current NASA grant (NAG-9-572):

1) To compile and analyze digitized databases (pre-flight, during flight and post-flight) of continuous ECG recordings from de-identified crew members from U.S. Spacelab Life Sciences and Shuttle missions and Russian Mir missions and to store these databases on compact discs (CD-ROM format) to facilitate distribution and retrieval.

2) To quantify the loss of complex heart rate variability as a potentially useful index of cardiac deconditioning during space flight, and during microgravity simulations with bedrest, in order to assess the effects of countermeasures such as LBNP and exercise.

3) To correlate the distinctive low frequency (<.01 Hz) heart rate oscillations observed during space flight with a) subjective motion sickness symptoms; b) activity level; and c) a respiratory signal derived from the Holter ECG.

4) To further develop a new nonlinear model of heart rate control and to incorporate gravitational effects to understand the mechanism of the observed dynamics.

These extensive, ongoing data analysis studies of spaceflight and microgravity simulation are being conducted in collaboration with NASA scientists at Johnson Space Center and Ames, and with Russian space scientists at the Moscow Center for Biomedical Research.

We have made progress in a number of complementary areas related to the specific aims of this task over FY 96:

(1) We have continued to develop and refine new algorithms for quantitating complex dynamical properties of heart rate time series, such as those obtained in space flight and in microgravity simulations.

(2) We have also continued to develop a nonlinear conceptual framework for understanding the types of complex dynamics observed during space flight and during microgravity simulations.

(3) We are working to expand our prototype database. This is the first prototype CD-ROM with heart rate data from the Mir Study as well as US astronaut data and bedrest 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.

(4) We have also developed new ways of quantitating human gait dynamics in health and disease. This work should have important practical applications in assessing the effects of space flight and microgravity on neuromuscular stability.

(5) A spin-off application has been the development of new algorithms based on statistical physics useful in the analysis of coding vs. non-coding DNA.

This research project seeks to understand two important conditions: space motion sickness and deconditioning associated with bedrest and microgravity. This research is directed at developing new ways of detecting and monitoring these conditions. These techniques promise to have general applications to clinical monitoring and to detecting patients at high risk of cardiopulmonary instability including life-threatening conditions. Furthermore, this work has led to new techniques for monitoring human gait in health and disease. This work has also led, as a spin-off, to new techniques for analyzing coding vs. non-coding DNA.

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Exercise Within LBNP to Produce Artificial Gravity

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Funding:

Project Identification: 199
Initial Funding Date: 10/95
FY 1996 Funding: $250,000

Solicitation: 93-OLMSA-07
Expiration: 9/96
Students Funded Under Research: 18

Responsible NASA Center: ARC

Task Description:

Calculations suggest that exercise in space to date has lacked sufficient loads to maintain musculoskeletal mass. Lower body negative pressure (LBNP) produces a force at the feet equal to the product of the LBNP and body cross-sectional area at the waist. Supine exercise in 100 mm Hg LBNP improves tolerance to LBNP and produces forces similar to those occurring during upright posture on Earth. Using a broader waist seal, LBNP at 50-60 mm Hg generates normal 1-G 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 1-G. Also, daily supine treadmill running in a LBNP chamber will maintain aerobic fitness, orthostatic tolerance, and musculoskeletal structure and function during bedrest (simulated microgravity). For bedrest 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 1-G. Second, bedrest 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 bedrest studies. Pre- and post-HDT tests will include orthostatic tolerance, cerebral blood flow, plasma volume, circumferences of body segments, peak oxygen uptake, leg muscle strength, GI function, and

gait analyses. We expect that supine LBNP treadmill exercise at one body weight will provide an accurate simulation of cardiovascular and musculoskeletal loads experienced while upright in 1-G. We further expect that 40 minutes of supine treadmill running per day in a LBNP chamber will maintain aerobic fitness, orthostatic tolerance, and musculoskeletal structure and function during 14 days of bedrest. The goals and objectives did not change during the course of our two years of NASA support.

The overall goal of this research project is to determine whether treadmill exercise within lower body negative pressure (LBNP) can simulate cardiovascular and musculoskeletal effects of gravity, and in doing so help prevent the physiologic deconditioning normally associated with bedrest and space flight.

The promising results from the five-day bedrest study in FY95 led us to perform a two-week bedrest study with a more strenuous interval exercise protocol (40-80% of peak VO_2) and a more comprehensive battery of pre- and post-bedrest tests. We increased daily LBNP exercise duration to 40 minutes and footward force to 1.0-1.2 body weights, but the 5 min of static LBNP after each LBNP exercise session and the comparison to daily upright exercise training were omitted. Seven male subjects acted as their own controls, such that their responses to two weeks of bedrest with daily supine LBNP treadmill exercise were compared to their responses to two weeks of bedrest with no daily exercise. Their participation in the two bedrest studies was separated by 10 weeks.

Forty min per day of LBNP exercise preserved upright exercise capacity during two weeks of bedrest. Subjects' time to volitional exhaustion during their individualized treadmill tests decreased 1.72 min on average (10%, $p < 0.05$) after bed rest with no daily exercise; daily LBNP exercise during bedrest maintained exercise tolerance time at pre-bed rest levels. Daily LBNP exercise also maintained peak upright VO_2 at pre-bedrest levels (pre-bed rest: 59.5 ± 3.2; post-bedrest: 56.4 ± 3.4). Mean peak VO_2 decreased from 57.6 ± 2.6 to 49.8 ± 1.5 ml x min-1 x kg-1 (14%) after bed rest with no exercise. Both respiratory exchange ratio (RER) and heart rate were consistently elevated at three sub-maximal running speeds relative to pre-bed rest measurements. Mean post-bedrest ventilation rate was significantly elevated at the highest two sub-maximal running speeds (13% and 17%) relative to pre-bedrest measurements. None of these effects were seen during sub-maximal exercise after bed rest with daily LBNP exercise: responses equaled those observed prior to bedrest. Sprint speed from a standing start was maintained at pre-bed rest levels when daily LBNP exercise accompanied bed rest (pre-bedrest: 5.5 ± 0.2 m x s-1; post-bedrest: 5.2 ± 0.3). However, bedrest without daily LBNP exercise reduced sprint speed from 5.5 ± 0.2 to 4.6 ± 0.3 m x s-1, or 16% below pre-bedrest control levels. During a walking test on a narrow rail, subjects walked longer following bedrest with daily exercise than after bed rest without exercise. Calf concentric and eccentric muscle strength, as assessed by peak ankle joint torque, remained at control levels after bedrest with daily LBNP exercise, and isometric strength actually tended to increase with the LBNP exercise treatment ($p = 0.19$). However, apparent reductions in plantarflexor strength after non-exercise bedrest were not statistically significant ($p > 0.07$).

Two weeks of bedrest reduced orthostatic tolerance 24% as assessed by LBNP (time to presyncope), and an essentially identical reduction in tolerance was seen after bedrest with daily LBNP exercise. Supine hematocrit increased from 39.7 ± 1.2 hematocrit units to 42.3 ± 0.9 (2.6 units, $p < 0.05$) after two weeks of non-exercise bedrest, yet no significant increase was seen in hematocrit after bedrest with LBNP exercise. Two weeks of bedrest tended to reduce plasma volume ($p = 0.25$), and LBNP exercise during bedrest appeared to counteract this effect to some extent, although these trends were not statistically significant. Results include data from one subject who exhibited an anomalous increase in plasma and blood volume after two weeks of bedrest without exercise. Exercise during bedrest increased fluid intake on average 413 ml per day (22%) relative to non-exercise bed rest conditions. Partial results ($n = 4$ — Dr. Lakshmi Putcha is completing analyses of our second bedrest study results) suggest a large delay in mouth-to-cecum transit time during bedrest, indicating a decrease in gastrointestinal motility. LBNP exercise maintained transit times associated with gastrointestinal function at pre-bedrest levels, whereas subjects exhibited slower transit times during bed rest without LBNP exercise. Five of the seven subjects reported that daily LBNP exercise improved the quality of their sleep during bedrest.

Results from this two-week bedrest study clearly indicate that 40 min per day of supine LBNP exercise at 1.0-1.2 body weights and 40-80% of peak VO_2 maintains upright exercise function and other normally

deconditioned variables at pre-bedrest levels. Why did daily LBNP exercise apparently protect orthostatic tolerance in our previous 5-day bedrest study (Watenpaugh et al., 1994b), yet did not protect tolerance in the two-week study? Use of 5 min of *static* (resting) 50-60 mm Hg LBNP following LBNP exercise in the previous investigation provides one possible explanation for the different findings of the two studies. Static upright posture or LBNP after strenuous exercise probably provides a substantially greater orthostatic stress than does the same stimulus after resting, non-exercise conditions. Therefore, imposition of several minutes of static, resting LBNP after the LBNP exercise session, as was done in our previous 5-day bedrest study, may restore the protective effect of LBNP on orthostatic tolerance which we observed in that prior study while still keeping the total countermeasure session to less than one hour.

Alternatively, mechanisms of orthostatic intolerance after two weeks of bedrest may be fundamentally different than mechanisms of orthostatic intolerance after five days of bedrest. For example, it is possible that hypovolemia alone explains predominantly post-bedrest orthostatic intolerance in the shorter study, whereas other mechanisms (baroreflex resetting, cerebral vascular acclimation to chronic cerebral hypertension, vestibular acclimation to simulated microgravity) predominate after two weeks of bedrest. Therefore, while daily LBNP exercise largely alleviates hypovolemia seen within a few days of bedrest, the exercise may not by itself adequately counteract all features of longer-term acclimation. Thus, we have added a 10 min period of nonexercise LBNP at 50 mm Hg to our exercise regimen in this renewal proposal.

The above results taken together strongly support continued development of LBNP exercise as a cost-effective alternative to centrifugation for periodic simulation of gravity and preservation of 1-G function during long-term existence in microgravity.

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 bedrest 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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Baroreflex Function in Rats after Simulated Microgravity

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Funding:

Project Identification: 199-14-17-17
Initial Funding Date: 12/95
FY 1996 Funding: $170,333

Solicitation: 95-OLMSA-01
Expiration: 11/97
Students Funded Under Research: 1

Task Description:

Prolonged exposure of humans to decreased gravitational forces during spaceflight results in a number of adverse cardiovascular consequences, often referred to as cardiovascular deconditioning. Prominent among these negative cardiovascular effects are orthostatic intolerance and decreased exercise capacity. Rat hindlimb unweighting is an animal model which simulates weightlessness, and results in similar cardiovascular consequences. Cardiovascular reflexes, including arterial and cardiopulmonary baroreflexes, are required for normal adjustment to both orthostatic challenges and exercise. Therefore, the orthostatic intolerance and decreased exercise capacity associated with exposure to microgravity may be due to cardiovascular reflex dysfunction. Our studies test the general hypothesis that hindlimb unweighting in rats results in impaired autonomic reflex control of the sympathetic nervous system. Specifically, we hypothesize that the ability to reflexly increase sympathetic nerve activity in response to decreases in arterial pressure or blood volume will be blunted due to hindlimb unweighting. There are 3 specific aims: 1) to evaluate arterial and cardiopulmonary baroreflex control of renal and lumbar sympathetic nerve activity in conscious rats subjected to 14 days of hindlimb unweighting; 2) to examine the interaction between arterial and cardiopulmonary baroreflex control of sympathetic nerve activity in conscious hindlimb unweighted rats; and 3) to evaluate changes in afferent and/or central nervous system mechanisms in baroreflex regulation of the sympathetic nervous system. These experiments will provide information related to potential mechanisms for orthostatic and exercise intolerance due to microgravity.

ATTENUATED BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY IN HINDLIMB UNWEIGHTED RATS.

This study tested the hypothesis that hindlimb unweighting in rats, an animal model of microgravity, results in attenuated baroreflex control of sympathetic nerve activity. Following 13 days of HU or normal cage activity, rats were implanted with femoral catheters and electrodes for recording either renal sympathetic nerve activity (RSNA) or lumbar sympathetic nerve activity (LSNA) and allowed to recover 24 hours. Thus, there were four groups of rats: control RSNA (n = 8), HU RSNA (n = 8), control LSNA (n = 8), and HU LSNA (n = 8). Reflex changes in RSNA or LSNA and heart rate (HR) were recorded in response to changes in arterial pressure. Mean arterial pressure (MAP) was increased or decreased by ramp infusions of phenylephrine and nitroprusside, respectively. Data relating RSNA or LSNA and HR to MAP were fit to a sigmoid logistic function, and curve parameters generated. Resting MAP was not altered by HU, while HR was significantly increased (HU: 423.8 ± 10.5, C: 365.4 ± 7.3). Maximal RSNA in response to decreases in MAP (HU: -5.1 ± 0.2, C: -15.0 ± 4.0)

were significantly reduced. In addition, maximal LSNA in response to decreases in MAP (HU: 204 ± 12% control, C: -7.8 ± 1.3) were also signficantly reduced. Baroreflex control of HR was not different between groups. Thus, HU attenuated baroreflex control of both RSNA and LSNA. These data are consistent with the concept that impaired baroreflex function could be a contributing factor to orthostatic intolerance following exposure to microgravity.

EFFECTS OF HINDLIMB UNWEIGHTING ON BARORECEPTOR AFFERENT SIGNALING.

Previous studies utilizing the HU model of microgravity indicate a significant attenuation in baroreflex control of sympathetic nerve activity. This experiment tested the hypothesis that the difference in baroreflex function is due to altered central processing of baroreceptor information, and not to an impairment of baroreceptor afferent responses to changes in pressure. Rats were either hindlimb unweighted (n = 4) by attachment of a tail harness, or served as cage controls (n = 4). Following 13 days of HU or control activity, rats were anesthetized with Inactin, and implanted with arterial and venous femoral catheters. Electrodes placed on the aortic depressor nerve (ADN) and on a branch of the renal nerve for recording renal sympathetic nerve activity (RSNA). Changes in ADN activity and RSNA were recorded in response to increases and decreases in mean arterial pressure (MAP) due to ramp infusions of phenylephrine and nitroprusside, respectively. Data relating RSNA to MAP were used to assess overall barorelex function; data relating ADN activity to MAP were used to assess baroreceptor afferent function; and data relating RSNA to ADN activity were used to assess central processing of baroreceptor afferent information. All data were fit to a sigmoid logistsic function. Curve parameters were generated for each animal and averaged. As in the previous study, HU reduced the maximum activation of RSNA in response to a drecrease in arterial pressure. Hindlimb unweighting did not signficantly alter the ADN activity in response to changes in arterial pressure. The efferent RSNA response to changes in afferent activity was reduced by HU. Thus, the attenuation of baroreflex control of sympathetic nerve activity does not appear to be accounted for by changes in afferent signaling, thus indicating a possible central mechanism in this dysfunction.

EFFECTS OF HINDLIMB UNWEIGHTING ON THE RESPONSE TO HEMORRHAGE.

Initial studies indicated that baroreflex mediated activation of the sympathetic nervous system in response to a hypotensive challenge was attenuated by hindlimb unweighting. This preliminary study has begun to evaluate the functional significance of this alteration in reflex function by testing the hypothesis that hindlimb unweighting reduces the ability of an animal to defend arterial pressure against blood loss. Rats were either hindlimb unweighted (HU) (n = 3) by attachment of a tail harness, or served as cage controls (n = 3). Following 13 days of HU or normal cage activity, rats were implanted with femoral catheters and electrodes for recording lumbar sympathetic nerve activity (LSNA), and allowed to recover 48 hours. Control and hindlimb unweighted animals were then subjected to hemorrhage at a rate of blood removal of 1 ml/min, and hemodynamic responses monitored. Preliminary data suggest that the reflex increase in LSNA and the blood loss required to reduce arterial pressure to 50 mmHg is less in HU rats compared to controls. These data are consistent with the concept that impaired baroreflex function in HU rats is functionally relevant.

State Dependent Aspects of Cognition

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Project Identification: 199-08-17-65
Initial Funding Date: 6/95
FY 1996 Funding: $55,038
Joint Agency Participation: NIMH

Solicitation: US
Expiration: 5/97
Students Funded Under Research:

Task Description:

Additional information was not available in time for publication.

Vitamin D RDA from Supplement of Light

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Funding:

Project Identification: 199-18-17-21
Solicitation: 95-OLMSA-01
Initial Funding Date: 12/95
Expiration: 11/96
FY 1996 Funding: $189,912
Students Funded Under Research:

Task Description:

Although it has been generally recommended that the RDA for young and middle age adults for vitamin D is 200 IU (5 micrograms), This is only adequate as long as they are exposed to sunlight. This issue is of particular interest to NASA as they begin to develop plans for longer duration spaceflights for the space station program. The goal of this research program is to critically investigate the RDA for vitamin D for healthy, young and middle aged male and female adults. We also plan to investigate whether passive exposure to simulated sunlight can produce enough vitamin D in the skin to satisfy the body's vitamin D needs. This will be accomplished by: 1). Establishing the vitamin D daily requirement for healthy young and middle aged male and female adults by supplementing groups of adults with a vitamin D supplement of either 0, 200, 400, 600, or 800 IU of vitamin D2 each day beginning in October and ending in March. During this time the cutaneous synthesis of vitamin D is inadequate in the Boston area. The determination of circulating concentrations of vitamin D, 25-hydroxyvitaminD, parathyroid hormone and other parameters of calcium metabolism will be evaluated throughout the study. 2). Determining the effect of controlled exposure to simulated sunlight 3 times a week on circulating concentrations of vitamin D, 25-hydroxyvitaminD, parathyroid hormone and other parameters of calcium metabolism between October and March. Study results will provide NASA with information that will be valuable in determining the amount of vitamin D that astronauts should take during long duration spaceflights for the space station program. In addition, this study will provide information on the possible use of simulated sunlight on space station as a means of ensuring that astronauts will produce enough vitamin D to satisfy their bodies' needs. The ultimate outcome of this study should provide NASA with important information about how much vitamin D is necessary in the absence of sunlight to prevent vitamin D insufficiency and vitamin D deficiency during long-duration space flight.

Exposure to sunlight is the major source of vitamin D for humans. Since astronauts are not exposed to ultraviolet B radiation (290-320 nm), they are incapable of photosynthesizing vitamin D in their skin for their body's requirement. The goal of our program is to determine the amount of vitamin D that is necessary to maintain a normal vitamin D status as determined by the measurement of serum 25-hydroxyvitamin D. To determine whether vitamin D can directly influence intestinal absorption of calcium, a study was conducted whereby healthy young and middle-aged adults were given various doses of vitamin D, 25-hydroxyvitamin D or $1,25(OH)_2D$. The results demonstrated that very high pharmacologic doses of vitamin D of upwards of 50,000 IU/day was necessary to enhance intestinal calcium absorption. Whether this effect was due directly to vitamin D or its metabolism to 25(OH)D is under investigation.

For long duration space flight, it may be reasonable to incorporate a source of ultraviolet B radiation on the Space Station. To determine the amount of surface area and total time of exposure that is required to produce an adequate amount of vitamin D to satisfy the body's requirement, a study has been initiated whereby healthy young and middle-aged volunteers were exposed to simulated sunlight to either hands, face and arms or their whole body. Blood has been collected to determine circulating concentrations of vitamin D and 25(OH)D.

To better understand the fundamental role that sunlight has in producing vitamin D in skin cells, basic studies were conducted whereby cultured human skin cells were exposed to simulated sunlight. It was found that the precursor of vitamin D, 7-dehydrocholesterol, is located within the cell membrane. Upon exposure to simulated sunlight, it is photolyzed to previtamin D_3. Normally, previtamin D_3 exists in two isomeric forms that include the s-cis, s-cis and s-cis, s-trans forms. It is the thermodynamically less favorable s-cis, s-cis form that is converted to vitamin D_3. We found that because 7-dehydrocholesterol is principally in the membrane of the skin cell, that upon its exposure to UVB radiation, it is efficiently converted to the s-cis,s-cis previtamin D_3. This isomer, in turn, is rapidly converted to vitamin D_3. As vitamin D_3 is formed, there is a rearrangement in its structure which helps it exit the cell membrane into the circulation.

The results from the ongoing studies have not resulted in any new questions. The results, to date, demonstrate the importance of determining the amount of vitamin D provided by exposure to UVB radiation as a means of maintaining good bone health for astronauts who live in a sunless environment.

The results from the ongoing research has direct application to human health and disease on earth and in space. Since vitamin D is absolutely essential for the maintenance of a healthy skeleton, it is important to better understand how much vitamin D and how much exposure to ultraviolet B radiation is necessary to satisfy the body's vitamin D requirement. Vitamin D deficiency is endemic in the elderly. Vitamin D deficiency causes adult rickets (osteomalacia) as well as exacerbates osteoporosis. This can increase a person's risk for skeletal fractures. The results from the ongoing research should provide the population at large with important new information about the recommended daily exposure to sunlight or simulated sunlight as a means of providing them with their vitamin D requirement.

FY96 Publications, Presentations, and Other Accomplishments:

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Holick, M.F., Tian, X.Q., and Allen, M. Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic animals. Proc. Natl. Acad. Sci., 92, 3124-3126 (1995).

Holick, M.R. "Photobiology and noncalcemic actions of vitamin D" in "Principles of Bone Biology." Edited by: Raisz, L.G., Rodan, G.A., and Bilezikian, J.P. Academic Press: San Diego, Ch.32, pp 447-460, 1996.

Holick, M.R. Environmental factors that influence the cutaneous production of vitamin D. Am. J. Clin. Nutr., 61, 638S-645S (1995).

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Monitoring Physiological Variables with Membrane Probes

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Funding:

Project Identification: 199-04-17-15
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 8/96
FY 1996 Funding: $41,811
Students Funded Under Research: 1

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 anti diuretic 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.

Membrane probes were developed and tested for *in vivo* monitoring of interstitial fluid in the rodent head down suspension model of microgravity. These probes included subcutaneous ultrafiltration and microdialysis probes and a muscle microdialysis probe. These probes were previously validated for electrolytes and lactate. Additional *in vitro* recovery tests demonstrated that the probes could also be used for glucose and vasopressin. Glucose recovery from ultrafiltrate probes was 99% +/- 3%. Recovery from DL-5 microdialysis probe with 2 µL/min perfusion was 90%+/-10 %. Ultrafiltrate probe vasopressin recovery was 91%.

Eight rats with subcutaneous probes were monitored interstitial changes in sodium, potassium, chloride, lactate and glucose during a baseline, suspension and recovery period. Ultrafiltrate sodium was significantly elevated ($p = 0.02$) during head down suspension. When returned to the normal baseline position the average sodium level dropped significantly lower than the head-down tilt average ($p = 0.002$) and also slightly lower than the baseline level ($p = 0.05$). Average plasma sodium levels show the same trends as ultrafiltrate. In plasma the difference between baseline and recovery periods is not significant. The difference between baseline and head down tilt is significant at $p = 0.08$. Plasma sodium concentrations are slightly higher than ultrafiltrate concentrations . The differences are significant in the baseline ($p = 0.02$) and head down tilt positions ($p = 0.05$). The microdialysis averages show the same trends as the ultrafiltrate.

Ultrafiltrate and microdialysate potassium concentrations decrease from baseline levels of 3.7 meq/L (UF) and 4.0 meq/L (MD) to 3.5 meq/L(UF) and 3.4 meq/L(MD) and during suspension. The difference is significant in microdialysate samples. Suspension in a head down position results in a decrease in chloride in both subcutaneous microdialysis and ultrafiltration samples while plasma shows a slight elevation. During the recovery period plasma remains unchanged, subcutaneous ultrafiltration showed an increase, and microdialysis a slight decrease. Suspension increases the subcutaneous ultrafiltrate glucose levels as well as plasma lactate. During recovery, subcutaneous glucose levels again decrease to baseline levels.

To sample from muscle a microdialysis probe with a 1 cm fiber was developed. This was implanted in the biceps femoris muscle of the rat. Continuous sampling was done over a 3 day period. Analyte concentrations fluctuate considerably under baseline conditions. This may be related to muscle activity. The average level of glucose remains fairly constant through the 3 day test period. Lactate, however, shows greater fluctuations. This may reflect different levels of lactate production with different levels of activity. The average lactate concentration declines over the course of the implant. Initial higher levels may be due to implant injury.

Sensors which were developed for on-line monitoring of glucose and lactate were coupled with the microdialysis probes for real-time glucose and lactate monitoring. The sensors were recalibrated daily and lost about 25% of their activity over a four day test period. In order to increase analyte concentrations, intraperitoneal injections of glucose and lactate were used. To decrease glucose levels, intraperitoneal injections of insulin were used. Concentration changes in subcutaneous lactate and glucose were detected with the on-line sensors.

This project has demonstrated the possibility of using membrane probes in rodents to monitor physiological variables for extended periods of time. The utility of these probes in physiological studies of microgravity has also been demonstrated. The feasibility of developing on-line sensors has also been demonstrated and allows for the possibility of developing real-time automated monitoring systems which can be used in ground-base physiological research as well as in research and medical monitoring in space.

The membrane sampling probes developed and tested are proving to be effective for continuous monitoring of a number of analytes which are routinely monitored in hospital situations for many different human diseases and conditions. The most significant advantage of these probes is that they allow monitoring without removal of blood. They also remove the need for repeated punctures and/or vascular access. Premature infants are monitored frequently for electrolytes and glucose. Because of their small size the withdrawal of blood for monitoring creates medical problems, and these infants must often receive blood transfusions to replace blood taken for monitoring. Therefore, physicians must constantly weigh the benefits of close monitoring with the disadvantages of transfusion. We have already demonstrated that we can continually monitor rats weighing 300 g for these analytes. This method of monitoring does not result in blood loss, so infants can be monitored as frequently as necessary to insure good metabolic control. An additional advantage to this method is that it does not require vascular access which is difficult to obtain in these small patients. Because of the difficulty in obtaining blood from a vein, samples are frequently obtained by puncturing the heel of the infant. This is a painful procedure. Since it is carried out frequently, the procedure disturbs the sleep of these patients and might possibly lead to some future psychological problems. Use of probes to monitor these infants would make sampling easier for the staff and less painful for the patient. Continuous monitoring could prevent such problems as brain damage resulting from hypoglycemia. Burn patients are another group of patients who would benefit from monitoring by probes. These patients are also very unstable and require frequent monitoring.

Since the membranes of these probes allow only low molecular weight substances to pass into the sample they are free of pathogens. Use of these probes in individuals with blood-borne diseases, who need frequent samples taken, could decrease the risks to staff assigned to obtain and analyze the samples.

The use of probes and sensors for continuous monitoring of glucose could be one of the most useful outcomes of this research for human medicine. Diabetes is the major disease with glucose abnormalities. Glucose monitoring is a necessity for maintenance of good health and for the reduction of morbidity and mortality among diabetics. Most diabetics do insufficient monitoring because of the pain and inconvenience involved in repeated

blood sampling and testing. A painless, continuous monitoring system which could be developed as an extension of this research would decrease the morbidity and mortality among diabetics. Also, this would significantly reduce the $14 billion annual national medical cost of diabetes.

In addition, this monitoring system would greatly facilitate diabetes research using small animal models. Monitoring glucose in these animals is now limited by the volume of blood which can be obtained.

The one analyte that we have found to be different in subcutaneous tissue and plasma is lactate. For this analyte the probe will not be an effective substitute for blood. However, the probes do offer a new technique for studying the metabolic pathways involving lactate in skin and subcutaneous tissue. Previously there was no method of measuring differences in concentration of analytes *in vivo* different tissues. Therefore, membrane probes offer a method for studying metabolism in different tissues and obtaining better understanding of physiological and pathological processes.

FY96 Publications, Presentations, and Other Accomplishments:

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Neural Control Mechanisms and Body Fluid Homeostasis

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Project Identification: 199-18-17-16
Initial Funding Date: 4/95
FY 1996 Funding: $141,936

Solicitation: 93-OLMSA-07
Expiration: 3/98
Students Funded Under Research: 3

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 mono-amine systems (e.g., norepinephrine; serotonin) are critical for precise correction of fluid losses. Because both acute and chronic dehydration are associated with physiological stresses, such as exercise and sustained exposure to microgravity, the present research will aid in achieving a better understanding of how vital information is handled by the nervous system for maintenance of the body's fluid matrix which is critical for health and well-being.

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. In our initial functional studies, two groups of rats were employed (n=6-13/group). One group received kainic acid injected into the right and left nodose ganglia and the other a sham (control) injection. After surgery, they were adapted to individual cages and given access to 1.8% NaCl and to water from burettes fitted with drinking spouts. Late in the afternoon, animals in the two groups were depleted of sodium by administration of the diuretic, furosemide (10 mg/kg). Food and the 1.8% NaCl containing burette was removed from the cages and 18 hrs. later, both groups of animals were given access to 1.8% NaCl. In a 2-hr access test to both NaCl and water, there was no difference in intake of either fluid. That is, sham treated animals vs. kainic acid treated animals drank 3.4 ml and 4.0 ml of water, respectively, and 6.6 and 6.5 ml of 1.8% NaCl.

In order to determine if destruction of afferent vagal fibers have an effect on rapid onset of water and salt intake, the same groups of rats were tested with furosemide followed immediately by captopril (10 mg/kg and 4 mg/kg s.c., respectively). Following this treatment, water and 1.8 % NaCl were removed from the animals but returned in one hr. Cumulative intakes of both fluids were then recorded for two hrs. There were no differences in water or saline intake in the sham vs. kainic acid treated rats over the time of the two hr. test. Total fluid intake for the two groups was 10.0 ml in the control animals and 10.5 ml in the animals receiving excitatory amino acid treatment of the nodose ganglia.

Taken together, the results of both of these experiments suggests that removal of vagal afferents is not a sufficient treatment to impair induced-thirst and -sodium appetite responses and suggests that if these afferents are involved in mediation of these fluid-related control mechanisms, redundant systems are capable of compensating for deficits in this type of afferent input.

In previous work, we discovered that systemic administration of the a2-adrenergic receptor antagonist, yohimbine (3 to 9 mg/kg s.c.), produces both vigorous water and concentrated NaCl intake in rats. Yohimbine is known to cross the blood-brain barrier. Therefore, we wished to determine whether intracerebroventricular (icv) infusions of yohimbine would induce thirst and sodium intake. Yohimbine was infused intravenously (1 ml/min) in doses of 0 (vehicle control), 3.5 (LO), 7.5 (MED), and 9.5 (HI) mg/ml for 1 hr. The animals had access to both water and 2% NaCl solutions from graduated burettes for a total of 3 hrs. At the conclusion of an 1 hr infusion period, access to both NaCl and water continued for an additional 2 hrs. Yohimbine infusion significantly increased the intake of both 2% NaCl and water. Water intake for the LO, MED and HI groups was significantly greater than intakes shown by vehicle treated animals after 120 min. The HI and MED experimental groups showed dose-related increases in 2% NaCl intake after 105 and 75 min, respectively. The results of these experiments suggest that blockade of noradrenergic action on a2 receptors at a brain site(s) accessed from the ventricles induces drinking behavior and sodium appetite which collectively serve to expand extracellular fluid volume. Further studies aimed at determining that site of action in the brain will be conducted.

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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Assessment of the Effects of Chronic Microgravity on Ventricular Mass by Three-Dimensional Echocardiography

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-80-07-02
Initial Funding Date: 3/95
FY 1996 Funding: $116,297

Solicitation: 93-OLMSA-07
Expiration: 2/97
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, and 2) to provide to NASA a space-capable three-dimensional ultrasound imaging system designed for accurate, quantitative measurements of organ volume and surface area for use by ourselves and other investigators.

To study the mechanism of cardiovascular adaptation of ventricular mass to chronic microgravity it is essential to have a means to accurately measure change of ventricular mass in individuals. This capacity has not previously been available. Three-dimensional echocardiography has been developed and proven to be methodologically superior to two-dimensional echocardiography for measurement of left ventricular volume, mass, surface area, and ejection fraction. Recent work indicates that it is able to accurately measure mass change in individuals, whereas previous methods have been validated only for populations. Previous work suggests that cardiac mass may decrease in microgravity. It may contribute to post-flight orthostatic intolerance and decreased exercise capacity. The time course and degree of decrease of cardiac mass are unknown due to lack of adequate, accurate available data for long duration space flights. Adequate interventions and countermeasures cannot be evaluated until these data are available. Inflight assessment of cardiac mass will permit evaluation and alteration of countermeasures during the course of long duration space flights.

We hypothesize that: 1) left ventricular adaptive changes to chronic microgravity results in decreases of myocardial mass proportional to decreases in circulating blood volume, and 2) decreases attributable to decreased cardiac work, and these changes are reversible over extended periods of time on return to Earth gravity. Three-dimensional echocardiography will be used to obtain pre-flight, in-flight and post-flight data sets for reconstruction of the ventricle and computation of left ventricular volume, mass, and function. Paired T-test and repeated measured analysis of variance will be used to determine if significant change of these parameters has occurred. It is anticipated that the data will show, upon entry into microgravity, an initial increase in myocardial volume, then a rapid adaptation, normalization, and then a gradual decrease of myocardial volume to a new lower level of homeostasis with no further significant change in ventricular volume, mass, or function on long duration space flight. After return to earth gravity, we expect the data to show that there is a decrease in chamber and myocardial volume, then rapid adaptation and normalization of ventricular volume and a slow return of mass to pre-flight values. Confirmation of our hypothesis will assure that astronauts will not suffer any permanent adverse effect on left ventricular function or mass on long duration space flight.

To facilitate development of a 3D ultrasound scanner for use in the International Space Station a comparative study of spatial 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 have been written. Testing is uderway.

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.

Validation of Spectral Analysis as a Noninvasive Tool to Assess Autonomic Efferent Regulation of Cardiovascular Function

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Project Identification: 199-14-17-02
Solicitation:
Initial Funding Date: 5/93
Expiration: 5/96
FY 1996 Funding: $0
Students Funded Under Research: 19

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

A major focus of our program has been to develop a sensitive noninvasive procedure to quantify early weightlessness-induced changes in cardiovascular function or potential dysfunction. Forty studies of healthy young volunteers (10 men and 10 women, each studied twice) were conducted to determine changes in the sympatho-vagal balance of autonomic control of cardiovascular regulation during graded headward and footward blood volume shifts. Changes in sympatho-vagal balance were classified by changes in the mean levels and spectral content of cardiovascular variables and verified by changes in circulating levels of catecholamines and pancreatic polypeptide. Possible shifts in intra/extravascular fluid were assessed from changes in hematocrit and plasma mass density while changes in the stimulus to regulate plasma volume were determined from plasma renin activity (PRA). Autonomic blockade was used to unmask the relative contribution of sympathetic and parasympathetic efferent influences in response to 10 min each of 0, 20 and 40 mmHg lower body negative pressure (LBNP) and 15 and 30 mmHg positive pressure (LBPP). The combination of muscarinic blockade with graded LBNP and LBPP was used to evoke graded increases and decreases in sympathetic activity without parasympathetic contributions. The combination of beta blockade with graded LBNP and LBPP was used to produce graded increases and decreases in parasympathetic activity without beta sympathetic contributions. Finally, a combination of both beta and muscarinic blockades with LBNP and LBPP was used to determine the contribution from other, primarily alpha adrenergic, sources. Mean values, spectral analyses, and time frequency analysis of R-R interval (HR), arterial pressure (AP), peripheral blood flow (RF), stroke volume (SV) and peripheral resistance (TPR) were performed for all phases of the study. Skin blood flow (SF) was also measured in other studies and similarly analyzed. Spectra were examined for changes in three frequency regions [low 0.006 - 0.005 Hz (LF), mid 0.05 - 0.15 Hz (MF), and high 0.15 - 0.45 Hz (HF)]. The primary objective of the study was to indicate which changes in the 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 to quantify the vascular and extravascular fluid shifts evoked by LBNP and LBPP. 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 would be detected by spectral analysis and verified by circulating hormones.

Hematocrit (HCT), plasma mass density and plasma renin activity increased with muscarinic blockade and with LBNP, a response indicative of a plasma shift to extravascular spaces. Beta blockade alone or after muscarinic blockade had no effect on HCT or plasma mass density. With respect to intravascular fluid volume distribution, LBNP and LBPP produced sufficient upper body vascular fluid shifts to evoke appropriate autonomic regulatory responses. Catecholamines increased in response to LBNP and pancreatic polypeptide (PPP) increased in response to LBPP.

In men, at rest and at all levels of LBNP and LBPP, muscarinic blockade resulted in higher mean values of HR, AP, TPR and hand vascular resistance with concomitant decreases in SV, RF, CO and end diastolic volume. The effect of beta blockade was to decrease HR and AP in control and at all levels of LBNP and LBPP. Either beta or muscarinic blockade given alone, resulted in a decrease in AP during LBNP that was either small or not present in the unblocked LBNP cases.

In the resting state, HR spectral power in all frequency ranges was only slightly affected by beta blockade, but was much diminished by muscarinic blockade. The heart rate response to LBNP was dominated by parasympathetic withdrawal in that the ratios of low to high frequency spectral powers, LF/HF, and mid to high, MF/HF powers were increased by LBNP and were unaffected by beta blockade. In the situations designed to evoke unopposed sympathetic and parasympathetic stimulation and withdrawal to regulate HR, we found that: 1) sympathetic stimulation resulted in an increase (with respect to resting control) in the (LF + MF)/HF spectral power ratio, with no changes in HF power, 2) sympathetic withdrawal resulted in a decrease in (LF + MF)/HF power ratio and a slight increase in HF power, 3) parasympathetic withdrawal resulted in an increase (with respect to resting control) in the (LF + MF)/HF power ratio and a large decrease in HF power, and 4) parasympathetic stimulation resulted in no change in (LF + MF)/HF power ratio or HF power.

In women, mean AP increased with either beta or muscarinic blockades. With muscarinic blockade alone, the increase in AP was due mostly to HR with a slight increase in TPR (SV decreased). With beta blockade alone, the increase in AP was due solely to TPR (HR decreased, SV did not change). The addition of beta to muscarinic blockade brought AP back toward control. Unblocked AP was well controlled during LBNP and LBPP: During LBNP, AP was maintained by increases in HR and TPR that countered the decreases in SV. After muscarinic blockade, the decreases in SV and the increase in TPR were slightly greater. After beta blockade, the decrease in SV and 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 data from women (1994 Progress Report) were compared with those from men (1993 Progress Report), the following differences were observed:

HORMONAL: 1) Men had significantly higher ($p<.0001$) levels of hematocrit than did women indicating lower relative plasma volume in men. The lower relative plasma volume was not due to a reduced signal to retain plasma since men had slightly higher levels of PRA than did the women. 2) In the unblocked state, men had greater levels of PPP than did women. After muscarinic blockade the PPP level of men dropped to equal that of women. 3) Men had greater levels of epinephrine ($p<.01$) than did women, and slightly higher levels of norepinephrine indicating greater sympathetic dominance in men.

MEAN VALUES: 1) Men had slightly lower unblocked HR (61 bpm) than women (67 bpm). After combined autonomic blockade HRs were 84 and 85 bpm, respectively, indicating that even though the intrinsic HR was the same, men had greater parasympathetic input in the unblocked state, perhaps to counteract the effects of the greater sympathetic activity. The balance of HR was however tilted toward sympathetic dominance in these men (see above). 2) Women had slightly lower (78 mm Hg) unblocked AP than men (83.5 mm Hg). After combined autonomic blockade, both pressures came to the same value (84 mmHg) via increased TPR in women (HR and SV changes offset each other).

The data collection has been completed; abstracts will be/have been presented at meetings and manuscripts are published or are being prepared. We have verified that results of spectral analysis of cardiopulmonary variables provide more sensitive indicators of autonomic balance than do mean values in healthy young men and women (abstracts and gender manuscripts in bibliography). We have further verified the sensitivity of these techniques to discriminate cardiovascular responsiveness to: 1) simulated weightlessness (discrimination manuscript) , 2) footward fluid shifts (abstracts), 3) headward fluid shifts (abstracts), 4) impending syncope (abstracts) and 5) susceptibility to syncope (abstracts). Results from these studies served as a basis for seven proposals: one each in response to NASA's 1994, 1995 and 1996 NRA, one in response to the DOD RFP concerning military women's health, two to NIH, and one to NASA EPSCoR.

Our research group participated 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 are being applied to patients with unexplained syncope. 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 26 patients with a diagnosis of unexplained syncope and 15 controls have been tested in a tilt test protocol using the 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.

FY96 Publications, Presentations, and Other Accomplishments:

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Adaptation in Artificial Gravity Environments

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Project Identification: 199-16-17-11
Initial Funding Date: 2/94
FY 1996 Funding: $373,542

Solicitation: 93-OLMSA-07
Expiration: 1/97
Students Funded Under Research: 9

Task Description:

The objective of the proposed research is to provide a technical base for evaluating the feasibility of a rotating "artificial gravity" environment for long duration space missions. We have previously demonstrated that Coriolis forces generated during rotation at 10 rpm disrupt head and arm movements but adaptation is possible. Here we will study 1) the rotation rates up to which adaptation is possible, 2) whether measurements of disruptions caused by rotation and subsequent adaptation in 1-G underestimate or overestimate the effects to be expected during rotation in environments with a background force level less than 1-G, and 3) how the magnitude and orientation of the background force affect retention and transfer of adaptation to rotation. Our studies will 1) result in recommendations regarding design criteria for artificial gravity environments, 2) provide sound scientific reasons for establishing confidence limits on the recommendation, 3) provide a basis for designing preadaptation procedures to alleviate expected problems in a rotating space vehicle, and 4) enhance basic understanding of spatial orientation on Earth.

Our goal is to understand how Coriolis forces that are generated by body movements disrupt eye-hand coordination and how to alleviate or prevent these disruptions. We have found that Coriolis forces disrupt the paths and endpoints of goal directed reaching movements. Subjects allowed repeated movements rapidly adapt, even in the absence of visual feedback about their reaching accuracy. This past year we have found 1) partial intermanual transfer of adaptation to Coriolis forces occurs; endpoint adaptation, but not path adaptation, transfers; 2) subjects allowed visual feedback about reaching accuracy show the same initial magnitudes of path and endpoint deviations but adapt more rapidly than subjects denied visual feedback; 3) subjects exposed to Coriolis forces in the weightless phase of parabolic flight maneuvers show smaller deviations of movement path and endpoint than when tested in one-G, and adapt more slowly with additional movements; and 4) head movements and leg movements are also deviated by Coriolis force perturbations. Leg movements adapt in the same fashion as reaching movements, but head movement endpoints and curvatures adapt to asymptotic values less than halfway back to baseline in darkness. We have extended our observations on postural stabilization by light fingertip contact with stable surfaces, and shown such cues: 1) override proprioceptive misinformation from leg muscles evoked by muscle vibration, 2) stabilize both head movement and postural control in labyrinthine-defective subjects. We are pursuing these findings to aid individuals with balance disorders, and astronauts with re-entry disturbances.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Cohn, J.V., DiZio, P., and Lackner, J.R. (abstract) Reaching movements during illusory self-rotation show compensation for expected Coriolis forces. Soc. for Neurosci. Abst., 169.19 (1996).

DiZio, P. and Lackner, J.R. (abstract) Reaching trajectory and endpoint errors induced by Coriolis force pertubations in labyrinthine-defective subjects. Soc. for Neurosci. Abst., 169.18 (1996).

Jeka, J.J., Easton, R.D., Bentzen, B.L., and Lackner, J.R. Haptic cues for orienttion and postural control in sighted and blind individuals. Perception & Psychophysics, 58(3), 409-423 (1996).

Jeka, J.J., Oie, K.S., Henson, E.M., Dijkstra, T.M.H., Schoner, G., and Lackner, J.R. (abstract) Somatosensory coupling to postural sway velocity. Soc. for Neurosci. Abst., 670.11 (1996).

Lackner, J.R. and DiZio, P. Motor function in microgravity: movement in weightlessness. Current Opinion in Neurobiology, 6, 744-750 (1996).

Lackner, J.R., DiZio, P., Jeka, J.J., and Rabin, E. (abstract) Fingertip contact suppresses the destabilizing effects of leg muscle vibration. Soc. for Neurosci. Abst., 641.8 (1996).

Money, K., Lackner, J.R., and Cheung, R. "The automatic nervous system and motion sickness" in "Vestibular Autonomic Regulation." Edited by: Yates, B.J. and Miller, A.D. CRC Press, (1996).

Motor Adaptation to Coriolis and Contact Forces

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Funding:

Project Identification: 199-16-17-05
Initial Funding Date: 2/94
FY 1996 Funding: $373,542

Solicitation: 93-OLMSA-07
Expiration: 1/97
Students Funded Under Research: 9

Task Description:

A rotating space vehicle could be used to generate "artificial gravity" during long duration missions, but this would have side effects. Movements made during body rotation would generate transient Coriolis forces that act perpendicular to both the rotation axis and the movement direction. We have found that such Coriolis forces initially deviate the trajectories and endpoints of reaching movements but adaptation occurs to restore accuracy if exposure continues. The patterns of movement deviation generated by Coriolis forces differ from what has been observed when movements are perturbed by external, local contact forces of comparable timing and magnitude. This implies that cutaneous contact cues are critical in the control and monitoring of movement endpoint, trajectory, and adaptation. Our new goal is to investigate the conjoint influence on reaching and adaptation of cutaneous sensory signals, proprioceptors and efferent commands. We will measure the effect on reaching movements of exposure to contact force perturbations, non-contact Coriolis forces and combinations of the two. Acquisition, retention and transfer of adaptation will also be studied. The results will allow us to refine our model of the adaptation, planning and execution of reaching movements. This will provide a basis for anticipating and solving potential performance problems in a rotating artificial gravity environment.

Our previous work showed that: 1) Coriolis forces generated by voluntary reaching movements in a rotating artificial gravity environment deviate the endpoints of those movements and make formerly straight paths curved. 2) Adaptation is possible within 20 reaching movements during rotation at 10 rpm. 3) What most influenced subsequent work was the additional finding that endpoint and path adaptation are complete and rapid if contact cues are permitted between fingertip and target surface but only curvature adapts fully if such contact is denied. In the last year we further investigated the combined roles of cutaneous and proprioceptive influences on movement control in artificial gravity and we found: 1) Individuals lacking somatosensation due to neuropathy of large myelinated sensory fibers show larger deviations of reaching due to Coriolis forces, they also show some straightening of their movement trajectories with practice. 2) Congenitally blind subjects adapt their movement trajectories and endpoints to Coriolis force perturbations as rapidly and fully as normal subjects tested blindfolded. In these experiments, subjects point to target positions on the work surface judged to be in their median plane. 3) Labyrinthine defective subjects show diminished ability, relative to normal subjects, to adapt to Coriolis force perturbations of their reaching movements when permitted terminal contact. The trajectories of their reaches become straight again but take longer to do so. Their movement endpoints show only partial adaptation and remain displaced in the direction of the prior acting Coriolis forces. 4) We perturbed reaching movements with contact forces applied to the arm at a single point but otherwise mimicking Coriolis forces. The contract forces deviated movement paths as much as Coriolis forces and endpoints half as much. With

continued reaches, adaptation to contact forces occurs but takes longer than for Coriolis forces. The first movement made without contact forces from the mechanical arm showed mirror-image deviations to the original perturbed movements, but half the amplitude. In additional experiments, we applied different patterns of contact forces to find the one which best reproduces the effects on arm movements of inertial Coriolis forces. We are currently running experiments to evaluate whether adapting to such optimal contact force perturbations will confer adaptation to Coriolis forces. This would provide a means of pre-adapting astronauts to a rotating artificial gravity on earth by means of a simple mechanical device.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Cohn, J.V., DiZio, P., and Lackner, J.R. (abstract) Reaching movements during illusory self-rotation show compensation for expected Coriolis forces. Soc. for Neurosci. Abst., 169.19 (1996).

DiZio, P. and Lackner, J.R. (abstract) Reaching trajectory and endpoint errors induced by Coriolis force perturbations in labyrinthine-defective subjects. Soc. for Neurosci. Abst., 169.18 (1996).

Jeka, J.J., Easton, R.D., Bentzen, B.L., and Lackner, J.R. Haptic cues for orientation and postural control in sighted and blind individuals. Perception & Psychophysics, 58(3), 409-423 (1996).

Jeka, J.J., Oie, K.S., Henson, E.M., Dijkstra, T.M.H., Schoner, G., and Lackner, J.R. (abstract) Somatosensory coupling to postural sway velocity. Soc. for Neurosci. Abst., 670.11 (1996).

Lackner, J.R. and DiZio, P. Motor function in microgravity: movement in weightlessness. Current Opinion in Neurobiology, 6, 744-750 (1996).

Lackner, J.R., DiZio, P., Jeka, J.J., and Rabin, E. (abstract) Fingertip contact suppresses the destabilizing effects of leg muscle vibration. Soc. for Neurosci. Abst., 641.8 (1996).

Money, K., Lackner, J.R., and Cheung, R. "The automatic nervous system and motion sickness" in "Vestibular Autonomic Regulation." Edited by: Yates, B.J. and Miller, A.D. CRC Press, (1996).

Spatially Oriented Database for Digital Brain Images [Human Brain Project]

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Funding:

Project Identification: n/a

Initial Funding Date: 10/95

FY 1996 Funding: $20,000

Solicitation:

Expiration: 6/98

Students Funded Under Research: 2

Joint Agency Participation: NIH and Human Brain Project

Task Description:

The overall goal of this project is the conceptual development and prototype implementation of a database methodology that supports the archiving and statistical investigation of large numbers and types of brain images. The specific aims of the study are: 1) to develop a morphologically factored image representation (MFIR) system that allows improved comparison of brain images, 2) to develop a Brain Image Database (BRAID) that supports novel statistical analyses of image data sets, and 3) to evaluate the database by applying it to both simulated data and to real data from 3 current brain imaging studies.

The MFIR is based on a nonlinear registration of an image to a standard atlas to create a morphologically normalized signal component and a morphological variation component, represented as a displacement vector field in atlas coordinates. The BRAID will implement storage, query and statistical operations on the MFIR components. The BRAID will be validated by testing its ability to recover known correlations from simulated data, and applied to the analysis of data from several collaborating epidemiological studies. The applications will test the system's ability to identify brain structure/function correlations from lesion/deficit data derived from stroke and injury, and its ability to identify patterns of morphological change in brain anatomy with age, and correlate these with functional data. Stroke data will be provided by the Cardiovascular Health Study, a National Heart, Lung, and Blood Institute sponsored project that is collecting extensive prospective demographic, functional, and brain Magnetic Resonance Imaging data on over 3,600 participants. Injury data will be collected by the Psychopathology of Frontal Lobe Injury in Childhood study, which is collecting brain MRI and extensive psychiatric/functional data on 100 children with traumatic brain injuries. Aging-related morphological and functional change data will be supplied by the Baltimore Longitudinal Study on Aging, which follows 180 patients over a nine year period and performs MRI and Positron Emission Tomography scans along with neurofunctional evaluations, on an annual basis. The newly developed database is intended to be flexible in terms of acceptable data types, robust in its querying mechanisms, and extendable to other laboratories, thus providing the basis of a future broad-based, multi-institutional brain informatics network.

We have explored methods for analyzing lesion/deficit correlations in a spatially-oriented database of brain image data. The data include 3-D descriptions of segments from MRI, as well as patient neurological function

variables. We have searched for correlations between all structures and all functions using 2 x 2 contingency table analysis with Chi Squared and Fisher tests. Significant correlations were detected which in most cases matched neurological expectations. We have also investigated methods of generating correlation images, including color-coding the p-values of the contingency analyses by structure. Such images can be readily generated by SQL queries against the augmented Illustrated database.

We have also explored atlas-independent methods of identifying brain regions significantly associated with a deficit. We used simulated annealing to optimize the size, position, and logistic regression parameters of a sphere which best discriminates between patients that have or do not have a particular deficit. Neurologically reasonable regions of interest were identified.

This research will provide insights into the localization of brain functions and the effects of stroke on brain function, and on the changes that occur in the structure and function of the brain with age. It will also evaluate a novel technology, extensible object/relational DBMSs, in an application which bears some relationship to geographic information systems and remote sensing databases. The object/relational technology enables an integration of data type-specific operations with general purpose relational ones. When the datatypes are spatially oriented, the result will hopefully be more powerful spatial database technologies.

Altered Brain Vasoregulation in Orthostatic Intolerance

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Funding:

Project Identification: 199-14-17-11
Initial Funding Date: 2/95
FY 1996 Funding: $242,369

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 5

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 studies of 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 and 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 bedrest. It evaluates the mechanisms, including brain mechanisms, and couples that with an evaluation of methods of treating the problem. We approach treatment with evaluating resistance training coupled with physical countermaneuvers. The studies of the brain, and in particular the brain stem ultra-slow rhythms, detectable on amplitude modulation of the EEG, may provide

important understanding of brain-stem mechanisms in regulating BP. The studies, by attempting to unify mechanisms of orthostatic intolerance on Earth and in space, provide a self-reinforcing approach to link space and Earth. The clinical applications of the research are potentially highly significant. It may result in a new way to treat orthostatic intolerance, as well as new methods to recognize it. The approach that we have adopted is unique in several respects. We have developed new algorithms, hitherto unavailable, to evaluate signals (time-frequency analysis, amplitude modulation of the EEG), and a combined approach in treatment of using physical countermaneuvers and resistance training.

FY96 Publications, Presentations, and Other Accomplishments:

Bouvette, C.M., McPhee, B.R., Opfer-Gehrking, T.L., and Low, P.A. Role of physical countermaneuvers in the management of orthostatic hypotension: Efficacy of biofeedback augmentation. Mayo Clin. Proc., 71, 847-853 (1996).

Fenton, A.M., Pieper, S., Low, P.A., Hammill, S., and Shen, W. Assessment of preload condition in patients with neurocardiogenic syncope: A potential mechanism for the augmented cardiopulmonary baroreceptor response. 16th Ann. Sci. Session of NASPE.

Low, P.A. Update on the evaluation, pathogenesis and management of neurogenic orthostatic hypotension. Neurology, 45 (Suppl. 5), S4-S5 (1995).

Physiological Transport Responses to High Intensity Exercise and Hydrostatic Pressure Gradients in Humans

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Funding:

Project Identification: 199-14-17-08
Initial Funding Date: 4/94
FY 1996 Funding: $92,000

Solicitation:
Expiration: 4/97
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 several important aims of our NASA grant related to mechanisms of plasma volume expansion following intense exercise. Overall, we have characterized the movement of albumin and fluid into the vascular compartment during the 24 hours following intense exercise. During the earlier phase

of plasma volume recovery (first two-three hours), varying hydrostatic pressure gradients (upright versus supine posture) had little impact on plasma volume recovery or the distribution of albumin in the vascular compartment. During the later phases of recovery (24 hour) from intense exercise in the upright posture, the increase in intravascular albumin content and subsequent expansion of intravascular volume was associated with a reduction in transcapillary exchange rates of albumin. However, in the supine position, the increase in intravascular albumin content and plasma volume expansion are prevented. Indirect estimates of capillary pressure in the forearm (using a rapid arterial occlusion technique) demonstrates a shift in the slope of the relationship between venous pressure and estimated capillary pressure following intense exercise. A change in the slope of this relationship reflects an alteration in the ratio of pre-to-post capillary resistance providing indirect support for a change in capillary hydrostatic pressure following intense exercise which would contribute to a retention of fluid within the vascular compartment. These data have been reported during the past year in abstract form.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Haskell, A., Nadel, E.R., Stachenfeld, N.S., Nagashima, K., and Mack, G.W. Forces governing transcapillary protein and fluid flux in human muscle and skin twenty-four hours after intense exercise. Physiologist, 39(5), A42 (1996).

Mack, G.W. Value of a high body fluid volume during physical activity. Physiologist, 39(5), A65 (1996).

Nagashima, K., Haskell, A., Nishiyasu, T., Mack, G.W., Cline, G.W., and Nadel, E.R. The effect of posture on exercise-induced hypervolemia. Physiologist, 39(5), A52 (1996).

Molecular Mechanisms Regulating IGF-I Synthesis in Bone

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-26-17-13
Initial Funding Date: 4/95
FY 1996 Funding: $135,495

Solicitation: 93-OLMSA-07
Expiration: 4/98
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.

Period of performance (year 2) - April 1, 1996 to March 31, 1997

This grant proposal has three specific aims. Specific Aim #1: Determine the DNA segments within promoter 1 of the rat IGF-I gene that confer sensitivity to stimulation by PGE2 in fetal rat osteoblasts (Ob). Specific Aim #2: Characterize nuclear protein factors from Ob cells that interact with basal and PGE2 response elements within the IGF-I promoter. Specific Aim #3: Determine if a cause and effect relationship exits between mechanical force, PGE2 (and cAMP) induction, and IGF-I promoter utilization.

Specific aim #1 has been accomplished, and the results published in the September 1995 issue of *Endocrinology* (McCarthy, T.L., Thomas, M.J., Centrella, M., and Rotwein, P.: "Regulation of insulin-like growth factor I transcription by cyclic 3¢,5¢-monophosphate (cAMP) in fetal rat bone cells through an element within exon 1: Protein kinase A dependent control without consensus cAMP response elements." (136):3901-3908). Briefly, we 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; and (3) co-transfection of IGF-I

promoter-luciferase-reporter constructs with a plasmid encoding a 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 next extended this observation and identified the minimal sequence needed for inducible binding at the HS3D footprint region, as tested in the gel mobility shift assay using nuclear protein extracts prepared from control and PGE2 treated osteoblast cultures, and transfection with mutant IGF-I promoter 1 constructs. This non-consensus cyclic response element (CRE) is 5'-CGCAATCG-3' and spans the +202 to +209 bp region of exon 1. Point and linker scanning mutations have been introduced into the HS3D footprint region, and both transient transfection and gel mobility shift analyses corroborate the importance of this sequence in PGE2 stimulated IGF-I expression. These data were presented at the 10th International Congress of Endocrinology, and were published in The Journal of Biological Chemistry vol. 271, pp 21835-21841, in a manuscript entitled "Identification of the cAMP response element that controls transcriptional ativation of the insulin-like growth factor-I gene by prostaglandin E2 in osteoblasts."

Research related to Specific Aim #2 is currently in progress. Nuclear protein extracts prepared from control and PGE2 treated primary rat osteoblast cultures were tested in gel mobility shift assays for binding to the novel CRE present in exon 1 at the HS3D footprint site. Initial characterization was accomplished by recovery of the PGE2 induced gel shift bands, followed by UV cross-linking of 32P-labeled oligonucleotide, to examine the relative molecular mass of the bound protein(s) on a denaturing SDS-PAGE. Results indicate a protein of <42 kDa binds to the HS3D site in nuclear extracts from PGE2 treated cultures. Preliminary searching using the Wisconsin Sequence Analysis Package (GCG) sequence database indicated some homology with several viral promoter elements that have similarity with CAAT element binding protein (C/EBP) sites. Gel mobility supershift analysis was conducted using a pan antibody to C/EBP that recognizes multiple isoforms of this transcription factor. A supershift was observed, indicating binding of C/EBP to the HS3D site present in the synthetic ds oligonucleotide. Using a C/EBPd specific antibody, we identified the reactive nuclear protein as the d isoform of C/EBP. Additional studies employing rat C/EBPb and C/EBPd expression vectors and co-transfection with IGF-I promoter constructs demonstrated augmentation of both basal and PGE2 induced IGF-I promoter activity by C/EBPd, and enhanced PGE2 induced IGF-I promoter activation with C/EBPb co-transfection. While we have already published that new protein synthesis is not required for PGE2 to elevate IGF-I mRNA expression, we did discover that PGE2 treatment elevated steady state transcript levels for both the b and d isoforms of C/EBP within 1 hr of treatment. Furthermore, this inductive effect of PGE2 on C/EBP transcript levels occurs in the absence of new protein synthesis. These new data have been accepted for an oral presentation at the upcoming annual meeting of the Endocrine Society this June in Minneapolis, MN. The abstract is entitled "CCAAT/enhancer binding proteins (C/EBP) mediate transcriptional activation of the insulin-like growth factor-I (IGF-I) gene by prostaglandin E2 (PGE2)." A manuscript is in preparation detailing these latest findings.

Specific Aim #3 involves testing the effect of mechanical strain on the expression of IGF-I promoter activity. This line of research examines mechanical strain applied to our primary osteoblast cultures using an Flexercell® FX-3000. Cultures are plated on collagen-coated flexible bottom cluster dishes, transfected with active IGF-I promoter construct (IGF1711b/Luc) when they achieve ~60-75% confluence, grown to confluence, then tested for their response to low amplitude and low frequency mechanical strain of varied duration. Control cultures are plated on comparable plates and experience no mechanical strain or are treated with an optimal dose (1 mM) of PGE2 to demonstrate a positive response to this IGF-I transcriptional activator (positive control). Preliminary data indicate a weak response to 0.1 Hz mechanical strain detectable within 6 hr of initiation. Further testing of varied amplitude, frequency, and duration are needed to optimize the response. In addition, our data indicating that PGE2 elevates the expression of both b and d isoforms of C/EBP has prompted us to examine the effect of PGE2 preconditioning of osteoblast cultures prior to initiating mechanical strain. A low dose (1 to 10 nM) PGE2 treatment will be tested for 6 or 24 h for its ability to augment the response to mechanical strain.

The high level of endogenous IGF-I synthesis by bone cells and its anabolic effects on bone indicate a major role for this factor in normal bone physiology. Locally produced IGFs are thought to participate in coupling bone formation to bone resorption. Therefore, it is important to understand the mechanisms bone cells utilize to regulate IGF-I activity. It is clear that IGF-I synthesis by osteoblasts is hormonally regulated. However, far less is presently known about the molecular mechanisms that regulate IGF-I expression. The loss of bone mass resulting in osteoporosis, seen in astronauts following exposure to microgravity and in older individuals, is thought to result from an imbalance between bone resorption and bone formation. In this vein, it is possible that a decrease in IGF-I synthesis resulting from a decrease in mechanical stimuli (in microgravity, or extended bedrest due to illness), or changes in hormonal status (post-menopausal, in aging, or in microgravity) may occur and limit the amount of available biologically active IGF-I. Reduced IGF-I levels may in part be responsible for uncoupled bone remodeling.

The effects of microgravity may be influenced directly by locally produced agents (prostaglandins, growth factors), and long-term skeletal defects may result from the indirect effects of changes in hormonal status (and subsequent changes in local growth factor actions). These are contributing factors that may be common to various forms of osteoporosis and disuse osteopenia, and even the associated bone loss observed in cases of trauma and immobilization, such as in severely burned individuals. Therefore, a thorough understanding of the mechanisms that regulate IGF activity in skeletal tissue is crucial to develop a more complete picture of normal bone physiology and may provide the means to augment bone matrix synthesis and to minimize or reverse the bone loss that results from the debilitating effects of microgravity induced and other forms of osteoporosis.

FY96 Publications, Presentations, and Other Accomplishments:

McCarthy, T.L., Shu, H., Ji, C., Casinghino, S., and Centrella, M. 17ß-estradiol potently suppresses cAMP induced insulin-like growth factor-1 (IGF-1) gene activation in primary rat osteoblast cultures. J. Bone Mineral Res., 11 Suppl. 1, S160 (1996).

Thomas, M.J., Umayahara, Y., Centrella, M., Rotwein, P., and McCarthy, T.L. (abstract) Transcriptional activation of the insulin-like growth factor-1 (IGF-1) gene by prostaglandin E2 (PGE2) in osteoblasts is controlled by a novel DNA element. 10th International Congress of Endocrinology (1996).

Thomas, M.J., Umayahara, Y., Shu, H., Centrella, M., Rotwein, P., and McCarthy, T.L. Identification of the cAMP response element that controls transcriptional activation of the IGF-1 gene by prostaglandin E2 in osteoblasts. J. Biol. Chem., 271, 21835-21841 (1996).

Vignery, A. and McCarthy, T.L. The neuropeptide calcitonin gene related peptide stimulates insulin-like growth factor 1 production by primary fetal rat osteoblasts. Bone, 18, 331-335 (1996).

Environmental Constraints on Postural and Manual Control

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Funding:

Project Identification: 199-16-11-48
Initial Funding Date: 2/95
FY 1996 Funding: $216,000

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 2

Responsible NASA Center: JSC

Task Description:

Extravehicular activity (EVA) is pivotal in supporting shuttle and station operations, including maintenance, construction, and contingency tasks. A ground-based investigation on the Precision Air-Bearing Floor (PABF) will provide information to determine the most efficient and safe methods for performing mass-handling tasks that are characteristic of ongoing EVA (e.g., Hubble repair and retrofit missions) as well as planned EVA (e.g., space station assembly and maintenance). This investigation will promote a better understanding of the whole-body skill of extravehicular mass-handling and, thus, it will help crewmembers convey their knowledge and experience about EVA for the purposes of training and planning for future missions. The investigation will also promote a better understanding of simulator fidelity with respect to extravehicular mass-handling and thus, it will suggest improvements or developments in simulators used to train and plan for future missions. Finally, the direction of the investigation has been modified slightly to expedite the development of measurement techniques that can be used on-orbit for more rapid evaluation and more efficient debriefing of EVA.

A "Yaw-Axis Cradle (YAC)" was designed and constructed for use with the extravehicular mobility unit (EMU) in the recumbent position on the PABF. The PABF-YAC allows us to investigate the skill of managing the postural mobility/stability tradeoff concurrently in orthogonal rotational axes (i.e., pitch and yaw). Data were collected in the PABF-YAC from EVA-experienced and -inexperienced subjects in a simulation of an extravehicular battery-box replacement. An EVA-experienced crew member participant in the investigation indicated that the experimental task was similar to many situations he had experienced while performing on-orbit EVAs. Communication has been established with the Advanced EVA Projects Office to enhance further the operational validity of the investigation and to facilitate coordination with other EVA investigations. Data also were collected in a "shirt-sleeve" study in which subjects stood upright and unrestrained while performing an otherwise comparable mass-handling task. These data were collected to evaluate the utility of exploring orbital replacement unit (ORU) "math properties" or handling qualities in shirtsleeve conditions. Unique analytical techniques have been developed to quantify essential aspects of mass-handling skill. These techniques address the adaptive coupling within the ORU-crew-member-EMU-restraint complex as a nested control system. They have been designed to reveal (a) the nesting of time scales for observation and control of both the ORU and the EMU, (b) the relation between the inertia tensor for mass handling and postural-manual coordination, (c) the

implications for mass-handling strategies, (d) the implications for various restraint systems, and (e) the implications for EVA simulations.

It has been determined that information about the coupling in the nested EV-crew member system is available in data collected with common instruments (e.g., video and force transducers). Analytical methods have been developed that reduce raw data from convenient instruments to higher-order measures of postural-manual coupling. These measures are sufficiently robust to be applied to low-observability situations such as ORU-EMU coupling and, potentially, to be used on-orbit. In addition, the operational validity of the PABF experiments has been verified. Thus, recommendations about on-orbit applications will be appropriate. More specifically, the experiments have revealed that (a) the additional degree of freedom (DOF) of mobility in the PABF-YAC is exploited during mass-handling simulations, (b) multi-axis postural perturbations can result from mass handling, (c) crew members engage in manual exploratory behavior that either substitutes for limited visibility or that otherwise promotes smooth control of an ORU, (d) restraint systems have an influence on postural mobility and stability that is relevant to mass-handling performance, (e) there are individual differences in mass-handling strategies and skill, (f) there are individual differences in situation awareness or sensitivity to the consequences that portable foot restraint (PFR) positioning and postural configuration have for mass handling, and (g) task demands influence mass-handling strategies.

The following questions have emerged for the third year of the investigation and beyond: (a) To what extent can an EV crewmember guide an IV crewmember in positioning the manipulator foot restraint (MFR)? (b) Should mass-handling efficacy influence the choice of MFR vs. PFR in EVA planning? (c) Can mathematical models extrapolate PABF findings to determine if information about ORU-EMU coupling is available in the Weightless Environment Training Facility (WETF) given the water viscosity? (d) Can EVA-relevant ORU handling qualities be learned in shirt-sleeve conditions on the PABF? (e) Are improvements possible in PABF protocols for learning ORU handling qualities? (f) In what ways do results from the PABF investigation suggest improvements to VR simulation and training for EV mass-handling? (g) In what ways can results from the investigation suggest improvements in whole-body strength models for EV crew members? (h) In what ways are results from the investigation relevant to free-floating operations? (i) In what ways are results from the investigation relevant to reduced-gravity Lunar or Martian EVA? (j) Can quantitative measures for mass-handling corroborate, complement, or resolve ambiguity in crew member comments? (k) Can quantitative measures for mass-handling be used or applied on-orbit?

Data collection and the development of analytical techniques are complete, therefore the quantitative evaluation of mass-handling skill will become the predominant activity. The analyses will focus on explanations for the observed effects that (a) different restraint systems, (b) PFR position, (c) task demands, (d) individual differences in skill and experience, (e) exploratory behavior, and (f) multi-axis perturbations in the PABF-YAC have on mass-handling performance and the supporting whole-body strategies. The conclusions about these effects will provide a foundation for addressing the questions that have arisen during the first two years of the investigation (listed above). These questions provide the bridge to on-orbit investigations, and the answers to these questions will suggest on-orbit applications of the new knowledge about mass-handling skill.

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.

FY96 Publications, Presentations, and Other Accomplishments:

McDonald, P.V., Riccio, G.E., Layne, C.S., and Bloomberg, J.J. How to exploit reactive phenomena in space. Presented at the 3-day International Conference "Bernstein's Traditions in Motor Control." Penn State University (August 23-25, 1996).

Riccio, G.E. Environmental Constraints on Postural Control and Manual Control. Gary E. Riccio Associates Report, GER 96-112-5 (1996).

Gravity and Bone Growth

Principal Investigator:

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Funding:

Project Identification:
Initial Funding Date: 10/95
FY 1996 Funding: $ 190,340

Solicitation: 93-OLMSA-07
Expiration: 9/96
Students Funded Under Research: 5

Responsible NASA Center: ARC
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 second grant year, two centrifuge studies were completed to test the following hypothesis. The sequence downstream of initial signaling events includes activation of the rapidly responding nuclear proto-oncogenes associated with proliferation and differentiation (i.e., c-*myc*, c-*jun*, or c-*fos*), followed by increased expression of late responding genes associated with matrix production and maturation (i.e., transforming growth factor-ß [TGF-ß], type I precollagen [ColI], and alkaline phosphatase [AlkP]). Subsequent to matrix maturation, activation of genes associated with matrix mineralization (i.e., collagenase and osteocalcin) occurs. These events culminate in formation of new bone which can be detected as an increase in tissue and serum levels of corresponding matrix proteins as well as increased bone mineralization rates, bone mineral content, and bone mass and accurately describes how mechanical loading stimulates bone formation. We anticipated that if centrifugation increased the mechanical forces on bone, that increased steady state message levels should be obvious immediately upon stopping the centrifuge and that these levels would then decrease with time following centrifugation until they returned to control levels. We compared the data from the centrifuged rats with those obtained from hindlimb unloading and found that 7d centrifugation (2-G) with return to 1-G was similar to, but less extensive, than hindlimb unloading. That is, bone mineralization rates were suppressed, not increased, and mRNA levels of bone marker proteins were not increased as predicted. However, the soleus muscle mass (corrected for body weight) was significantly greater in the centrifuged animals compared to controls indicating that the postural muscles were sensing an increased load. Data analysis of these studies is continuing.

Preliminary interpretation of the data suggest that centrifugation more closely resembles hindlimb unloading than limb overloading. Unlike the hindlimb unloading studies, centrifuged animals tend to gain significantly less weight than controls. For this reason, a food restricted group was included as a body weight matched control group for the centrifuged animals. Even with food restriction and maintaining similar body weights, this control group gained more weight per gram of food consumed than did the centrifuged group suggesting different metabolisms in the two groups. Centrifugation did appear to increase muscle mass as soleus mass (corrected for body weight) did increase significantly in the centrifuged group compared to controls. Bone mineralization and bone area were significantly lower in centrifuged and food restricted animals as compared to ad libitum controls. Steady state message levels showed few changes in either cortical or cancellous bone within 5hr of stopping the centrifuge.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Evans, G.L., Morey-Holton, E., and Turner, R.T. (abstract) Spaceflight has compartment specific effects on mRNA levels for bone matrix proteins and bone matrix production in rat femur. J. Bone Mineral Res. 11 (Suppl. 1): S270, 1996.

Globus, R.K. and Morey-Holton, E.R. (abstract) Skeletal changes during hindlimb unloading of growing rats. International Workshop on Bone Research in Space, Tokyo, Meeting Abstracts, p. 15, November 1996.

Halloran, B.P., Bikle, D.D., Harris, J., Tanner, S., Curren, T., and Morey-Holton, E. (abstract) Cortical bone formation: Effects of skeletal unloading and intermittent administration of parathyroid hormone. ASGSB Bull., 10:59, 1996.

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Holmuhamedov, E.L., Moursi, A., Lull, J., Morey-Holton, E., Damsky, C., and Globus, R.K. (abstract) Laminin regulates differentiation and survival of cultured osteoblasts. 6th International Congress on Cell Bio. & 36th American Society for Cell Biology Annual Meeting Special Poster Session, December 11, 1996.

Malouvier, A.C.L., Zerath, E., and Morey-Holton, E. (abstract) Hypergravity prevents age-related trabecular bone loss in young rat tibiae. J. Bone Mineral Res. 11 (Suppl. 1): S269, 1996.

Morey-Holton, E.R. (abstract) Gravity and biology. NASA-Ames Research Center Astrobiology Workshop Meeting abstracts, September 1996.

Morey-Holton, E.R. and Arnaud, S.B. (abstract) Bone and calcium metabolism: The importance of gravitational loading and diet. Japanese Society of Space, Aviation, and Environmental Medicine, Meeting Abstracts, p. 31, November 1996.

Morey-Holton, E.R. and Arnaud, S.B. Bone and calcium metabolism: The importance of gravitational loading and diet. Japanese Society of Space, Aviation, and Environmental Medicine, Tsukuba, November 15, 1996, pp. 1-6.

Morey-Holton, E.R., Whalen, R.T., Arnaud, S.B., and Van Der Meulen, M.C. "The skeleton and its adaptation to gravity" in "Handbook of Physiology: Environmental Physiology." Edited by Fregly, M.J. and Blatteis, C.M. Section 4: The Gravitational Environment, Vol. 1: Microgravity. New York: Oxford University Press, Chapter 31, pp. 691-720, 1996.

Patterson-Buckendahl, P., Poppalardo, D., Kvetnansky, R., Globus, R., Bikle, D., Halloran, B., and Morey-Holton, E. (abstract) Opposing effects of vitamin D and stress hormones on bone osteocalcin concentration. J. Bone Mineral Res. 11 (Suppl. 1): S425, 1996.

van der Meulen, M.C., Beaupre, G.S., Morey-Holton, E.R., and Carter, D.R. (abstract) Diaphyseal bone growth and adaptation: Models and data. European Society of Biomechanics, 1996.

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Westerlind, K.C., Morey-Holton, E., Evans, G.L., Tanner, S.J., and Turner, R.T. (abstract) TGF-ß may help couple mechanical strain and bone cell activity *in vivo*. J. Bone Mineral Res. 11 (Suppl. 1): S377, 1996.

Effect of Gravity on the Regulation of Circadian Rhythms

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Funding:

Project Identification: 199-18-17-19
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 4/98
FY 1996 Funding: $112,765
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 affect rhythmicity, but it is not yet known if a change in gravity affects rhythmic functioning by acting directly upon the suprachiasmatic nucleus (SCN), which is the central neural pacemaker. These experiments will examine both the effect of a hypergravity on circadian function and the neural mechanism through which this action takes place. This will be accomplished by testing the hypotheses that exposure to two-G will depress both circadian rhythms and gene activity within the SCN and that recovery of rhythmicity will be correlated with recovery of gene activity in the pacemaker. Further, if two-G does act as a synchronizer for circadian rhythms, it will also entrain the expression of protein synthesis within the SCN.

During the second year of this NASA grant, several of the specific aims have been accomplished. Our previous studies have demonstrated that continuous exposure to two-G via centrifugation abolishes the circadian rhythms of heart rate, body temperature, and activity in rats for approximately two to three weeks. In addition, it was demonstrated during the first year of the grant that the loss of circadian rhythms following two-G exposure has a direct effect on the c-Fos activity within the neural pacemaker (i.e. SCN). Therefore, during the past year we have examined the effect of prolonged exposure of two-G via centrifugation on circadian rhythms and c-Fos activity within the SCN.

We have completed the examination on the effect of a 48 hour exposure to two-G on c-Fos expression within rat SCN neurons. There was a significant decrease in c-Fos expression within SCN neurons following two-G exposure relative to that of controls. These results demonstrate that the effect of two-G 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 two-G exposure. Control one-G 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 two-G, there was no increase in c-Fos expression in SCN neurons. These results further demonstrate that two-G exposure has a highly significant effect on the function of the neural pacemaker.

We have demonstrated that the recovery of c-Fos expression in the SCN of rats that have been exposed to continuous two-G via centrifugation for a period of three weeks. We showed that a recovery in c-Fos expression in the SCN coincided with the previously demonstrated recovery of circadian rhythms during two-G exposure. It was also shown that there was a recovery in the effect of a phase shifting light pulse to induce c-Fos reactivity in SCN neurons after three weeks of two-G exposure.

The results from the research to date compliment the planned experiments for FY97. Planned studies will examine the pattern of c-Fos recovery within the SCN by sampling different lengths of continuous two-G exposure (one hour, two days, one week, two weeks, and three weeks). In addition, it will be important to determine whether two-G exposure prevents the direct expression of c-Fos within SCN neurons, or if the RHT has become dysfunctional in conveying light information to the SCN. We will start to examine the effect of one hour two-G pulses to entrain circadian rhythms. We will also extend the examination of the effect of two-G exposure on c-Fos expression from the SCN to include other neural areas such as the hypothalamus, autonomic nervous system nuclei, and the vestibular and motor system nuclei. This will broaden our perspective of the effect of hypergravity on the CNS and provide a better perspective on the specific functional changes in the SCN.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Fuller, C.A., Hoban-Higgins, T.M., Murakami, D.M., and Tang, I-H. Effect of pre-natal exposure to microgravity on circadian rhythms in rats during the flight of NIH.R2. ASGSB (1996).

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Tang, I.-H., Murakami, D.M., Hoban-Higgins, T.M., and Fuller, C.A. The effect of flight NIH.R2 on retino-hypothalamic tract development. ASGSB (1996).

Fully Implantable Integrated Silicon Biotelemetry

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Project Identification: 199-80-07-03
Solicitation: 93-OLMSA-07
Initial Funding Date: 3/95
Expiration: 2/98
FY 1996 Funding: $233,338
Students Funded Under Research: 2

Task Description:

The primary objective of this project is the development of miniature, fully implantable multichannel biotelemetry systems for the measurement of physiological parameters. These low-power systems will transmit recorded information using an implanted transmitter, and are targeted to measure biopotentials, blood pressure, core body temperature, multi-axis acceleration, and pH in small unrestrained rodents and primates. These devices will eventually be used by NASA in its animal studies for both ground-based and space experiments.

Although the general direction of our research efforts still remains the development of a complete system, upon discussion with researchers at NASA Ames research center in August 1996, our efforts have been focused on two areas that are of critical need to NASA, as discussed below.

1) Long-term base-line stability is a major concern in implantable pressure transducers. Base-line drift in these sensors impede their use in chronic applications unless frequent calibrations are performed. These calibrations are time consuming and impractical when access to the animal is not possible. To overcome the shortcomings of current devices, a new approach of designing a miniature pressure sensor array using silicon micromachining technologies to account and compensate for any long-term drift has been taken and is currently under development. This will have a great impact not only in biotelemetry measurements in animals but also in many implantable medical devices that require chronic long-term pressure measurements. In addition to pressure sensors, this project also aims at the development and implantation of sensors for the measurement of multi-axis acceleration and neural biopotentials in unrestrained animals.

2) We will design and fabricate low-power, high-performance data acquisition and telemetry circuits to be used with various sensors in implantable telemetry units. These will include: biopotential amplifiers, switched-capacitor charge readout circuits for capacitive sensors, interface circuits for resistive sensors, low-power multiplexer and A/D converters, microcontroller, and bi-directional telemetry circuitry. These circuits can be fabricated through commercial foundries and will be available to various investigators. The development of standard circuit blocks that can be easily retrieved and used in implementing new biotelemetry systems will significantly reduce the development and fabrication time. These circuit blocks will be available to other potential users in the future, and it is the goal of this project to develop a library of these circuit blocks.

In the second year of this project, we have made important progress in several areas. We continued our efforts in the design, fabrication, and test of the capacitive pressure transducers. In order to interface these sensors to the outside, we designed and fabricated a switched-capacitor interface circuit. The problem of base-line drift in implantable pressure sensors is a formidable one. We are in the process of designing a pressure sensor array that can be used to offset any long-term drift by appropriate signal processing. We are planning to test these sensors for long-term drift at NASA Ames facilities in the coming months.

We have developed a generic micromachined silicon platform for low-power, low-loss miniature transceivers. This platform supports high-Q inductors and low-loss capacitors and resistors on suspended dielectric diaphragms. This platform can be used to build miniature transceivers, either stand-alone to support hybrid attached surface-mount transistors or flip-chip bonded to RF circuits on an integrated chip, thus saving valuable chip area. The transmitter is a Colpitts oscillator which is switched on and off by a one Mbps incoming digital data in a PCM format. All the passive components are fabricated on a silicon substrate and the only active part (Motorola RF transistor MRF931) is connected to the substrate. The micromachined platform is rugged and its fabrication does not depend on the silicon crystal orientation for selective substrate removal. It can support active hybrid components while reducing the large capacitances associated with their bond pads by suspending them on dielectric diaphragms. The integrated coil has five turns, each 25 μm wide, six μm thick, and separated by 25 μm. It is suspended on diaphragm patches 0.3x1.8 mm in dimensions. The inductance and quality factor of the coil at 200 MHz are 0.4 μH and 20 respectively. The tuning capacitor can be laser trimmed to set the transmission frequency to any value between 175-220 MHz. The quiescent current drain of the transmitter is 100 μA current with a 3 V driving voltage. The transmitter chip area is 5x5 mm^2.

We are now developing the circuit blocks of our fully integrated, single chip data acquisition system. These circuit blocks consist of instrumentation amplifiers, A/D conversion system, microcontroller, and telemetry unit. Instrumentation amplifiers for biopotentials (ECG, EEG, EOG, EMG) are being designed now. The most challenging problem associated with instrumentation amplifiers is the large dc offset compared to information bearing signals. In addition to the need for canceling this offset, they should possess highly linear amplification and pre-filtering to achieve high signal quality. We are working on the design of low voltage, low power instrumentation amplifiers which will have continuous-time pre-amplification stage with dynamic DC compensation followed by switched-capacitor filtering. Each amplifier will be fed into a micro power A/D conversion channel to avoid analog multiplexing which can degrade the system performance by increasing intermodulation and switching noise. A compact, micro power A/D conversion cell employing successive approximation method has already been designed and fabricated. This ADC provides eight-bit resolution, five Kbps conversion rate with 100 μW power consumption at three V supply voltage. The ADC circuits have been fabricated through the circuit fabrication foundry service of MOSIS using a 1.2μm CMOS process, and is currently undergoing extensive testing. In addition to the low voltage, low power, dynamically-switched analog CMOS front-end circuitry, we have also designed a microcontroller unit that operates the bi-directional telemetry unit, provides power management, and will provide the user access to the desired sensor configuration with programmable gain, bandwidth, and accuracy.

In summary, during the second year of this project, we have made progress in several areas which are of considerable importance to the implementation of miniature biotelemetry systems. These areas include high performance micro sensors, bi-directional telemetry module, and low-power interface circuitry for data acquisition. In the coming year, we will design and fabricate a miniature pressure sensor array for extra-vascular blood pressure measurement and a multi-axis accelerometer for monitoring physical activity. The pressure sensor array will overcome the drift problem associated with implantable transducers. We will also complete the design and fabrication of low-power data acquisition circuit blocks required for miniature implantable biotelemetry systems. These will include totally integrated low-power biopotential amplifiers, switch capacitor read-out circuitry for capacitive sensors, interface circuit for resistive transducers, A/D converter and microcontroller.

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.

Spacelab Rotating Chair Data Analysis

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Funding:

Project Identification: 199-70-17-20
Solicitation: 93-OLMSA-07
Initial Funding Date: 1/95
Expiration: 03/97
FY 1996 Funding: $0
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 four to five 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 five data sets (n=21 pre/post, n=12 inflight), plus data from three 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.

A paper describing these results has been publised in the Journal of Applied Physiology. During the past year, our efforts have focussed on reanalysis of the virtually identical SLS-1 and SLS-2 mission data using a common methodology. The results will be incorporated into the SM Thesis of Mr. Matt Neimark in January, 1997. So far, the analysis generally confirms the findings of SLS-2, including the significant inter-subject differences in the effects of prolonged zero-G on postrotatory time constants. The study also provides stronger statistical support indicating a difference between dumping VOR responses in weightlessness vs. 1-G, and the similarity of head erect vs. dumping responses in prolonged zero-G. Taken together, these findings imply that VOR nystagmus dumping depends on gravireceptor input. In addition, our results show that averaging across the entire group of 8 subjects, head upright VOR time constants have not yet returned to preflight values by the time of late postflight testing (R+5-10 days). VOR gain data showed no signficant trends. A manuscript describing the combined SLS-1/SLS-2 study, and also presenting an analysis of rotation sensation duration data obtained on SLS-2 is now in preparation.

The goal of this study is to better understand the influence of gravity cues on the human 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.

FY96 Publications, Presentations, and Other Accomplishments:

Oman, C. Sensory Conflict Theory and Space Sickness: Our Changing Perspective. J. of Vest. Res., (in press).

Oman, C. (abstract) The effect of prolonged weightlessness on the vestibulo-ocular reflex of astronauts. Annals of Biomedical Engineering, 23, Suppl. 1, p. S-89, Abstract 427 (1995).

Oman, C., Pouliot, C., and Natapoff, A. Horizontal angular VOR changes in orbital and parabolic flight: human neurovestibular studies on SLS-2. J. Appl. Physiol., 81(1), 69-81 (1996).

Oman, C. Pouliot, C., and Natapoff, A. Horizontal angular vestibulo-ocular reflex changes in orbital and parabolic flight. XIX Extraordinary Barany Society Meeting, Sydney, Australia (August 13, 1996).

Mechanisms of Sensorimotor Adaptation to Centrifugation

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Funding:

Project Identification: 199-16-11-54
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $200,000
Students Funded Under Research: 0

Responsible NASA Center: JSC

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 past year, we completed a study examining the spatial coding of eye movements relative to perceived head and Earth orientations during static roll-tilt (Wood et al., 1997). Results from this study suggest that spatial coding during roll-tilt is more accurate when eye movements are made relative to a perceived Earth reference frame and the axis of eye movement is aligned with gravity. Spatial coding of eye movements was then examined during roll-tilt stimuli induced with sustained centrifugation using our newly developed Short-Arm Centrifuge Facility. Preliminary results from this study suggest that spatial coding of eye movements is similar in 1-G and hyper-G environments. This new technique using spatially directed eye movements along perceived orientations will provide a new dependent tilt measure for future studies which we plan to conduct on tilt adaptation during sustained centrifugation.

We also examined the effects of roll-tilt on optokinetic nystagmus (OKN). This study failed to duplicate the results of other investigators, suggesting that OKN responses would reorient toward a gravitational frame of

reference during roll-tilt on Earth. Since a reorientation of the OKN responses to align with gravity would not be compensatory for the visual stimulus, there may be natural limitations on cross-coupling during OKN as it would presumably be beneficial to maintain alignment of eye movement response with the visual stimulus direction. The lack of a robust optokinetic after-nystagmus, which has been difficult to elicit in humans, may also suggest that the velocity storage elicited during our optokinetic stimulus was weak and therefore was not sufficient to drive a reorientation in the response axis. However, we did observe a reduction in the magnitude of the horizontal OKN during roll-tilt which may reflect the same spatial orientation function. We interpret this as follows. During natural horizontal head movements, the axis of head rotation and therefore optokinetic stimulation is normally aligned with gravity. During roll-tilt on Earth, the horizontal OKN is suppressed when the response axis is not aligned with gravity. One possible explanation for this is that otolith cues indicating a static tilt orientation are conflicting with visual cues which are signaling head rotation. Based on these preliminary results, we now plan to incorporate a head-fixed optokinetic stimulus during sustained centrifugation to provide a conflicting visual-vestibular stimulus. This additional visual condition will enhance our planned experiments for this next year, which are designed to examine the spatial reference frame used for coding orientation and motion as a function of exposure to sustained hypergravity tilt induced during short-radius centrifugation.

A set of companion experiments is ongoing using longer-radius centrifugation at the Naval Aerospace Medical Research Laboratory (NAMRL) in Pensacola, FL. These studies have examined the differences in the per-rotatory angular and linear vestibulo-ocular reflexes (VOR) during centrifugation as a function of backward/forward facing subject orientations and as a function of gaze direction (Raj et al., 1996). Preliminary data analysis is ongoing to compare eccentric yaw VOR records using a one m radius in our Short-Arm Centrifuge Facility at JSC with data recorded at 2 m and 6 m using the NAMRL centrifuge facility. These studies will be especially useful in evaluating differences between short- and long-radius centrifugation for future space applications with operational constraints which will limit the size of inflight centrifuge facilities.

In addition, this grant has supported further development of two models of sensory interactions which have been used to predict three dimensional VOR responses during complex motion stimulation involving hypergravity. Merfeld (1997) has applied internal models of sensory dynamics and body dynamics, and examined model predictions with and without a fixed magnitude for the internal representation of gravity. This model has been compared with the recent work of Lionel Zupan who applied "coherence constraints" to canal-otolith-vision interactions. Both models successfully predict the asymmetric VOR responses during backward/forward facing subject orientations we have observed in our centrifuge experiments without requiring a fixed (one-G) internal representation of gravity. Moreover, a new model of regular-irregular otolith unit dynamics has been recently implemented which permits better predictions of the LVOR component during eccentric rotation and the L-nystagmus component during lateral translation in the light (Merfeld, personal communication).

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.

FY96 Publications, Presentations, and Other Accomplishments:

Guedry, F.E. Spatial coding of eye movements relative to perceived head and earth orientations during static roll-tilt. Brain Res. Bull., 40, 505-512 (1996).

Merfeld, D.M. Vestibulo-ocular reflex of the squirrel monkey during eccentric rotation with centripetal acceleration along the naso-occipital axis. Brain Res. Bull., 40, 303-309 (1996).

Paloski, W.H., Reschke, M.F., and Wood, S.J. A static tilt device to evaluate adaptation to altered gravito-inertial force environments. Life Sciences and Space Medicine Conference, American Institute of Aeronautics and Astronautics, Houston, TX; March (1996).

Raj, A., Fajardo, A., Luke, B., Rupert, A., Benson, A., and Guedry, F.E. Effects of gaze on the human linear and angular vestibulo-ocular reflexes during centrifugation. J. of Vest. Res., 6, S23 (1996).

Perceived Self-Motion Assessed by Computer-Made Animations

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Funding:

Project Identification: 199-16-17-12
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $94,754
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.

Experiment 1

Data collection was completed. 36 subjects reported perceived self-motion following exposure to complex inertial-visual motion stimuli. eight subjects were assigned to each of three perceptual reporting procedures: (a) animation movie selection, (b) verbal report selection and (c) verbal report generation. The question addressed was: do reports produced by these procedures differ with respect to complexity and reliability? Following repeated (within-day and across-day) exposures to four different "motion profiles," subjects either (a) selected movies presented on a laptop computer, or (b) selected verbal descriptions from a booklet, or (c) generated self-motion verbal descriptions that corresponded most closely with their motion experience. 1) "complexity" and 2) reliability "scores" were calculated.

The means were essentially equivalent for animation selection and verbal report selection procedures: no statistically significant differences between reporting procedures were observed. The data suggest that reports by verbal report generation subjects were less complex than for the other conditions. The hypothesis that animation selection would be more reliable than the verbal report procedures was not supported.

Several reasons may account for the failure of this experiment to demonstrate clearly expected advantages of animations. First, appropriate, careful training to use a standard self-motion description vocabulary may eliminate possible differences between reporting procedures. Subjects may be better able to describe motion verbally than is usually believed. Second, the motions may not have been sufficiently complex. Based on the stimulus motions, only fairly simple self-motions (2 DOF translational and 1 DOF rotational) would be expected. Third, movies/verbal descriptions depicting combined scene and self-motion perception, which almost certainly corresponded with the subjects' actual experience, were not used. Fourth, individuals probably differ with respect to how they represent motion cognitively. Some may use pictorial representations, whereas others may use verbal descriptions.

Experiment 2

Experiment 2 is designed to answer the following question: do the perceptual reporting procedures yield equivalent or different data?

Experiment 3

The results from Experiment 1 suggest that failure to observe differences between reporting procedures may be due to the motion vocabulary training. The hypothesis that an animation procedure will produce more reliable data than a verbal report procedure if the motion vocabulary training is omitted will be examined in Experiment 3.

Because subjects cannot readily use the animation movies selection procedure without training, Experiment 3 will employ a new procedure: animation generation. This will be accomplished by having the subject manipulate a mannequin so that the mannequin's motion corresponds to the perceived self-motion. Polhemus Fastrak sensors embedded in the mannequin will permit "real-time" representation of the motion on a monitor as well as recording of that motion for later analysis.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Harm, D.L., Parker, D.E., Reschke, M.F., and Skinner, N.C. (abstract) Astronauts' microgravity "rest frame" selection correlates with pre- and postflight vection latencies. Life Sciences and Space Medicine Conference '96 - Book of Abstracts, Washington, D.C. American Institute of Aeronautics and Astronautics (1996).

Parker, D.E., Harm, D.L., Sandoz, G.R., and Skinner, N.C. (abstract) Perceived self-motion assessed by computer generated animations: sensitivity and reliability. Journal of Vestibular Research, 6, S16 (1996).

Prothero, J.D., Hoffman, H.G., Parker, D.E., Furness, T.A., and Wells, M.J. Foreground/background manipulations affect presence. Human Factors and Ergonomics Society 39th Annual Meeting. Santa Monica, CA. Human Factors Society (1995).

Facilitated Blood Pressure Control by Skin Cooling: Autonomic Mechanisms

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Project Identification: 199-14-17-15
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $0
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

This project has been temporarily suspended until Dr. Pawelczyk's duties as a Payload Specialist for STS-90 (Neurolab) are completed.

Relation of Motion Sickness Susceptibility to Vestibular and Behavioral Measures of Orientation

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Project Identification: 199-16-17-04
Solicitation:
Initial Funding Date: 9/93
Expiration: 9/96
FY 1996 Funding: $0
Students Funded Under Research: 0

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 vestibular reflex properties and behavioral responses with motion sickness susceptibility. Specifically we have measured vestibulo-ocular reflex (VOR) responses to a variety of motions designed to stimulate different vestibular motion sensors. Behavioral responses refer to postural control properties related to the integration of visual, vestibular, and somatosensory motion information required for maintenance of upright stance. We have completed the data collection phase and now have measures of VOR responses, postural control properties, and motion sickness susceptibility in 12 subjects.

Motion Sickness Susceptibility

Our primary stimulus for inducing MS is a constant velocity rotation of a subject in the dark with the subject's yaw axis (long body axis) tilted 30° from an Earth-vertical axis. This off-vertical axis rotation (OVAR) stimulates the vestibular otolith organs (linear acceleration sensors) since the subject's head position is constantly changing its orientation with respect to gravity. Due to the biomechanical properties of the vestibular semicircular canals (sensors or transient rotational motion), the canals are not stimulated by this stimulus. Therefore there is a "conflict" between motion information sensed by different portions of the subject's vestibular system. Specifically the otolith organs signal an ongoing dynamic tilting and/or rotation of the subject while the semicircular canals signal that no corresponding rotational motion is occurring. The presence of this sustained sensory conflict can evoke MS symptoms in susceptible subjects. The use of this particular stimulus is reasonable for a study attempting to relate its results to space MS, since it is postulated that conflicting motion information from the semicircular canals (whose function is presumably not altered in a zero-G environment) and otolith organs (whose function is altered in zero-G) is a likely initiator of MS experienced by astronauts.

All 12 of our test subjects were exposed to a 14-minute OVAR stimulus during which the progression of MS symptoms were monitored. Testing was terminated when subjects reached a moderate level of symptoms. Data from these tests will allow us to rank the MS susceptibility of the test subjects based on test termination times or the level of symptoms at the end of the 14-minute test. About half of the subjects completed the entire test with minimal symptoms while the other half showed a wide range of susceptibilities. This wide distribution should provide a good data set for comparison with VOR and postural control results.

Behavioral Responses - Postural Control

Our earlier results (published in *Exp. Brain Res.* 105:101-110, 1995) enhanced our knowledge of postural control behavior and allowed us to develop posture test stimuli that provide quantitative estimates of each individual's reliance upon visual, vestibular, and/or somatosensory orientation cues for balance control. There is a large variation among individuals in their preferred use of orientation cues from different sensory systems for postural control. 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 have completed data collection in our 12 subjects. The postural control testing exposed each subject to an extensive set of stimuli designed to characterize the extent to which the subject made use of visual, vestibular, and somatosensory information for postural control. Subjects stood on a platform facing a visual surround. Stimuli were provided by rotations of the platform and/or visual surround at various amplitudes and frequencies. Additional test conditions included eyes open/closed, and sway-referencing of the platform and/or visual surround. (Sway-referencing refers to a technique of rotating the platform or visual surround in proportion to the measured sway of the subject. This has the effect of largely eliminating visual and/or somatosensory motion cues that are

normally well correlated with body sway.) The main dependent variables are anterior-posterior body sway and center-of-pressure (COP) recorded from the platform in response to the various test stimuli.

We have completed the basic analysis of all our posture data. These results include calculation of body sway and COP amplitude and phase, and measures of average, peak-to-peak, and variance of body sway and COP. In addition we calculated various "stabilogram diffusion function parameters" in order to quantitatively characterize the properties of spontaneous postural movements.

Reflex Parameters - VOR

The dynamic properties of the VOR are indicative of both peripheral vestibular receptor function and central nervous system processing of vestibular motion information. Various preliminary experiments were used 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. Results of one of these preliminary studies comparing the dynamic response properties of vertical and torsional eye movements were presented at the Association for Research in Otolaryngology meeting in February 1996.

We 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 included an OVAR test that provides dynamic stimulation to the otolith organs following the decay of the VOR component due the semicircular canal stimulation. This OVAR test was similar to the one used to evoke motion sickness, but was shorter in duration.

We completed VOR testing in our 12 subjects and have begun analysis of these data. The analysis of eye movement data has been a "rate limiting" step in the overall study. Our eye movements were recorded using a custom developed video-oculography system that requires computer image analysis in order to extract relevant measures of horizontal, vertical, and torsional eye movements. With the aid of additional funds from a NIH/NASA-sponsored center grant "Otolith control of posture" (P60-DC02072), we are nearing completion of an updated and improved image analysis system that is more accurate and 60 times faster than the previous version. We are continuing to make changes to improve analysis speed and accuracy by taking advantage of new technology and of other researchers' contributions to the rapidly developing field of video-oculography. This new system will largely eliminate the rate-limiting step of analysis of video eye movement data.

As part of the video-oculography development, we have devised a novel calibration technique for video-oculography. This technique uses non-linear optimization methods to estimate the orientation of a video camera with respect to a subject's head. Accurate camera orientation information is necessary in order to make accurate 3D eye movement measurements. Our new technique appears to be more robust than older published methods, and was presented at the Barany Society meeting in Sydney, Australia (1996).

Normative VOR data from this grant also contributed to work performed as part of the NIH/NASA-sponsored center grant (P60-DC02072). The center grant study involved 8 patients with apparent severe bilateral loss. A comparison of our normative VOR responses to responses from bilateral loss subjects during yaw plane, pitch plane, and OVAR rotations suggest that otolith function often is preserved in patients with severe bilateral vestibular loss (as determined by conventional clinical testing) and that this preservation might contribute to a successful compensation for a bilateral vestibular deficit.

We have just started work on the recently obtained competitive NASA grant renewal. The first year of this grant involves making equipment modifications required for the proposed studies of dynamic postural control and adaptation. During this first year, we also will be completing the VOR data analysis and correlating VOR measures and postural control behavior with our motion sickness susceptibility data, and preparing papers for publication.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Peterka, R.J. Comparison of vertical and torsional vestibulo-ocular reflex response dynamics in humans. Association for Research in Otolaryngology, Mid-winter meeting, Florida (1996).

Peterka, R.J. and Merfeld, D.M. (abstract) Calibration techniques for video-oculography. J. Vest. Res. 6:S75 (1996).

Peterka, R.J., Horak, F.B., and Shupert, C.L. (abstract) Preservation of otolith function with bilateral vestibular deficits may contribute to compensation. J. Vest. Res. 6:S90 (1996).

Pulmonary Deposition of Aerosols in Microgravity

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Project Identification: 199-14-17-09
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $214,999
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.

In FY96, we successfully flew the total deposition portion of the proposed research on the NASA KC-135 Microgravity Research Aircraft. In the course of four flights during one week, we measured the total deposition of inhaled aerosol of sizes 0.5, 1, 2, and 3 mm, both in microgravity (μG) and hypergravity (~1.6G) in four subjects. These results were compared to measurements made in one-G. Data analysis indicates that excellent data were obtained, and clear, statistically significant results were obtained. A manuscript is in preparation for submission to the Journal of Applied Physiology in early 1997. We have now succeeded in performing the first ever extensive documentation of the behavior of the range of aerosols primarily affected by sedimentation. The results suggest that in μG, while larger particles are deposited less than in one-G, deposition of the smallest particles studied is in fact considerably higher than anticipated. This appears to be due to the non-reversible nature of the airflow in the human bronchial tree, which manifests itself as an apparent enhancement of Brownian diffusion. The consequence is that alveolar deposition of these small particles is higher than that predicted by existing models of aerosol behavior in the lung. It is apparent that existing models of aerosol deposition require modification in order to take into account the enhanced diffusion present in the small airways. This effect is always present, but it has not been apparent in the past, because of the large deposition due to sedimentation. Only by eliminating sedimentation, by performing the studies in μG, was it possible to clearly unmask this effect. The question that must now be answered is that of how great deposition is in the alveolar spaces because of enhanced diffusion. Since the alveoli are highly susceptible to damage by inhaled substances, this may have a fundamental bearing on the development of some environmentally based pulmonary diseases. For example, it is now believed that much of the rise in asthma prevalence may be due to the inhalation of

small (< 2.5 mm) particles, and new federal standards are being proposed to control the levels of these particles. The recent findings in our studies of total deposition emphasize the need for direct measurements of regional deposition and dispersion, studies using inhaled boluses of particles. We have already proposed these measurements as part of this program using one mm particles. Based on the findings of enhanced diffusion, we think it likely that we will wish to extend the range of particle sizes used to more accurately map the range of particle diffusion rates. In addition, we think it likely that a breathhold should be incorporated into the studies, especially for the smallest particle sizes. The inclusion of a breathhold will allow for increases in Brownian diffusion without increases in enhanced diffusion, since reciprocal flow due to respiration will be absent during the breathhold period. In addition we intend to increase efforts on the improvement of our existing models of aerosol deposition to enable them to better predict the behavior of the aerosol in μG. At present, we intend to follow our existing research plan, and proceed with the one mm bolus studies, as these form the first logical step in the study of enhanced diffusion. The first of these studies is scheduled to fly on the KC-135 in March 1997.

This program seeks to obtain a better understanding of the processes of deposition of inhaled particles in the human lung. Inhaled particles deposit on the walls of the airways and gas exchange regions of the lung by three mechanisms: impaction of large particles, sedimentation of medium sized particles, and movement by diffusion of the smallest particles. Particle deposition is important in many diseases that result from working in dusty environments, e.g., silicosis and asbestosis among many. Further, the deposition of particles in the lung is very important in the delivery of many therapeutic agents e.g. the metered dose inhalers used by asthmatics. In these cases, the site and efficiency of deposition of the medium sized particles is critically important for the efficacy of the drug therapy. Since sedimentation is a gravitational process, by studying the changes in deposition of test particles in the absence of gravity, we hope to gain a better understanding of the entire process of deposition. This can then be fed back to provide better aerosol generation, targeting more specific sites in the lung. The process of deposition in the weightless environment is also clearly important for the people that will be continuously exposed to suspended particles in the Space Station environment.

Mechanisms of Microgravity Effect on Vascular Function

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Funding:

Project Identification: 199-14-17-10
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $168,113
Students Funded Under Research: 2

Task Description:

The proposed study addresses the effects of microgravity on vascular function with particular relevance to the problem of orthostatic intolerance experienced by astronauts on re-entry following space flight. It is clear that the decreases in plasma volume and baroreceptor reflex responsiveness during space flight contribute to, but do not fully account for, re-entry orthostatic intolerance. The proposed study will investigate the largely unexplored possibility that adaptive changes in vascular smooth muscle and/or associated sympathetic or other innervating nerve terminals occur during space flight (zero gravity) that result in decreased responsiveness of the vasculature. Microgravity is simulated using the hindlimb unweighted (HU) rat, and the following vessels are removed from HU and paired control rats for *in vitro* analysis: abdominal aorta, carotid and femoral arteries, and jugular and femoral veins. Three mm-long rings of vessel are mounted in tissue baths for the measurement of either isometric contraction or relaxation of precontracted vessels. The isolated mesenteric vascular bed is perfused for the measurement of changes in perfusion pressure as an index of arteriolar constriction or dilation. The justification for this work is that it will explore a potential major mechanism underlying orthostatic intolerance, thereby providing a basis for the development of more effective countermeasures.

Experiments were designed to explore 1) the effect of simulated microgravity on vascular contractility to serotonin, 2) the contribution of the endothelium to the vascular effects of simulated microgravity, and 3) the time course of the onset of the vascular effects of simulated microgravity.

HU was used to simulate microgravity. 20-day HU was shown in the previous year of this study to reduce the maximal contractile response of all arteries studied to norepinephrine. In the present year, it was found that HU treatment had no effect on the response of the abdominal aorta, carotid, or femoral arteries to serotonin. This was further confirmed in aorta by obtaining contractile responses to norepinephrine and serotonin in separate rings from the same aortas of both control and HU treated rats. HU treatment depressed contractility of these rings to norepinephrine but not serotonin.

The role of the endothelium in the vascular effect of HU was explored in several ways. First, the endothelium was mechanically removed. This treatment had no effect in aorta and femoral artery. In contrast, endothelium removal abolished the depression of contractility to norepinephrine in the carotid artery. The role of the endothelium was explored further by testing the sensitivity of phenylephrine-precontracted arteries to the endothelium-dependent relaxing effects of acetylcholine and the endothelium-independent relaxing effects of

sodium nitroprusside. HU had no effect on the sensitivity of aorta and femoral artery to acetylcholine, but increased the sensitivity to acetylcholine in carotid artery. HU did not change the sensitivity of carotid and femoral arteries to sodium nitroprusside, but decreased the sensitivity of the aorta to this endothelium-independent relaxing agent. In an additional series of experiments, Western blotting was used to determine the effect of HU on protein mass of both endothelium constitutive nitric oxide synthase (ecNOS) and inducible nitric oxide synthase (iNOS), most likely located in the vascular smooth muscle. 20-day HU increased both ecNOS and iNOS in aorta, carotid, and femoral arteries.

The time course for the onset of HU effect was assessed by measurements made at 1, 3, 7, and 20 days. HU-induced reductions in contractility to norepinephrine were observed after one day in carotid artery, three days in aorta, and only 20 days in femoral artery. The HU-induced increase in sensitivity to the relaxing effects of acetylcholine in carotid artery were seen only at 20 days.

The present results demonstrate that increased NOS activity, producing the vasodilator nitric oxide, accounts, in part, for the depression of contractility induced by HU. However, the occurrence of such depression after only one day, taken together with the requirement 20 days to induce an increase in acetylcholine sensitivity in the carotid artery, points to additional mechanisms. The failure of HU to depress contractility to serotonin, in contrast to its effect on that to norepinephrine, points to an effect of HU at the level of calcium handling and/or earlier second messenger steps that mediate norepinephrine-induced contraction. Future experiments will explore the effects of HU on both NOS- and second messenger-related processes.

This research seeks to understand the malady of postural intolerance experienced by space-adapted astronauts on return to the Earth's gravitational field. This research will yield new information concerning biological mechanisms in the vascular system by which exposure to microgravity depresses vascular contractility, contributing to postural intolerance. It is the long-term goal of this research to use the new understanding of these biological mechanisms to develop specific therapies to prevent microgravity-induced postural hypotension and intolerance.

Concerning humans on Earth, there are several maladies in which the patient experiences postural intolerance or hypotension. These include dysautonomia, diabetes, and any malady that requires long-term, continuous bedrest. Some of the same mechanisms that underlie microgravity-induced postural intolerance may operate in these conditions as well. The specific therapies developed to help astronauts may also be found to be beneficial in these patients.

FY96 Publications, Presentations, and Other Accomplishments:

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Experimental Neurogenic Hypertension Program: Supplement

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Funding:

Project Identification: 199-08-17-72/P01HL18974-18-20 Solicitation:

Initial Funding Date: 9/94 Expiration: 8/96

FY 1996 Funding: $ Students Funded Under Research: 2

Joint Agency Participation: NIH/National Heart Lung and Blood Institute

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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. In this study, we investigate 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 1996 we continued the investigation of the RVL mechanisms governing hypoxic cerebral vasodilation. We established that in anesthetized paralyzed rats, the cerebrovasodilatory effect of microinjection of sodium cyanide (NaCN) (150-450pM) into the RVL is dose dependent. NaCN (150-450pM) microinjected into the RVL rapidly (1-2 sec), transiently, dose-dependently, and site-specifically elevated rCBF, as measured by laser-Doppler flowmetry, by 61.3±22.1% ($p<0.01$), increased arterial pressure (AP) (+30 ± mmHg; $p<0.01$), and triggered a synchronized six Hz rhythm of electroencephalographic activity. Following cervical spinal cord transection, NaCN, and also dinitrophenol, significantly ($p< 0.05$) elevated rCBF and synchronized the EEG, but did not elevate AP; the response to NaCN was attenuated by hyperoxia and deepening of anesthesia. Electrical stimulation of NaCN sensitive sites in RVL in spinalized rats increased rCBF, as measured autoradiographically with 14C-iodaoantipyrine (Kety method), in the midline thalamus (182.3±17.2%; $p<0.05$) and cerebral cortex (to 172.6±15.6%; $p<0.05$) regions, respectively, directly or not innervated by RVL neurons, and in the remainder of brain. In contrast, regional cerebral glucose utilization (rCGU), measured autoradiographically with 14C-2-deoxyglucose, was increased in midline thalamus (165.6±17.8%, $p<0.05$) in proportion to rCBF but remained unchanged in cortex. Bilateral electrolytic lesions of NaCN-sensitive sites of RVL did not affect resting rCBF nor its elevation elicited by hypercarbia ($PaCO_2$, ~69 mmHg); however, it flattened the slope of the PaO_2/ rCBF response curve and reduced the vasodilation elicited by normocapnic hypoxemia of PaO_2 ~27 mmHg by 67% ($p<0.01$).

In order to characterize cortical neurons that are involved in vasodilation evoked by activation of cyanide sensitive sites within RVL, we sought to identify cerebral cortical neurons whose activity related to spontaneous and neurogenically evoked elevations in rCBF and which, as established earlier, increased activity during hypoxia. Rats were anesthetized, spinalized and ventilated while recording cortical EEG, rCBF (laser Doppler flowmetry), pO_2 and neuronal activity. Rats displayed diffuse spontaneous synchronous bursts of EEG that were identical to a thalamically-driven burst-suppression EEG pattern and consisted of a constant triphasic potential with variable afterbursts invariably followed, ~1.2 sec later, by a monophasic elevation of rCBF paralleled by elevation in intracortical pO_2. Identical burst-cerebrovascular wave (CW) complexes were evoked by single electrical stimuli of cyanide sensitive sites within RVL or the cerebellar fastigial nucleus (FN) with latencies of 33 and 48 msec, respectively. Fifteen of 362 spontaneously active cortical neurons (4.6%) increased their discharge >two-fold ($p<0.05$) during the early negative wave of the triphasic potentials, whether spontaneous or evoked from FN or RVL, before the rise of rCBF. The neurons were located in cortical layers V-VI. When RVL or FN were stimulated continuously for 10 sec (50Hz), the activity of these neurons increased 2.4-fold ($n=6$; $p<0.05$) in advance of the sustained (5-8 min) elevation of rCBF, and declined in parallel with recovery of rCBF and reappearance of burst-CW complexes. The results indicate the presence of a small population of neurons in deep cortical laminae whose activity correlates with neurogenic elevations of rCBF. These neurons may function to transduce afferent neuronal signals into vasodilation.

We conclude that the elevation of rCBF produced in the cerebral cortex by hypoxemia is in large part neurogenic and results from excitation of oxygen-sensitive neurons of RVL, which, in their turn, excite a small population of cortical vasodilatory neurons in deep cortical laminae, coupling at least some forms of cortical neuronal activity to the associated elevations in rCBF.

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 re-entry to a terrestrial environment.

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The Sympathetic Nervous System in the Anemia of Weightlessness

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Funding:

Project Identification: 199-08-17-60
Solicitation:

Initial Funding Date: 3/94
Expiration: 3/97

FY 1996 Funding: $ 149,361
Students Funded Under Research: 5

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 bedrest, and systematic studies to assess the potential of simple countermeasures such as sympathomimetic amine preparations to correct the erythropoietin deficiency and anemia. These studies have potential implications for patients chronically at bedrest which may be similar to those for astronauts and, if our hypotheses are correct, may lead to changes in the management of anemia produced by chronic bedrest.

We are now finishing our final two subjects for this anemia and microgravity study. The study will then be completed. We have met our goals.

This research was undertaken in an effort to better understand how an absence of gravity might lead to anemia. This original question is considered relevant to patients at chronic bedrest. These studies are continuing, and we should understand the relevance of this to the anemia of chronic disease by the end of the study. The unexpected discovery that in patients with orthostatic intolerance (mitral valve prolapse, chronic fatigue syndrome, and other disorders which fall into this framework), there is a very significant increase in loss of fluid from the vasculature during upright posture. This observation, made possible by the NASA support of the anemia studies, may have important implications for the future management of patients with orthostatic intolerance. It is believed that approximately 500,000 Americans suffer from orthostatic intolerance. No mechanism for this has ever been clearly identified. The documentation in our study of a dynamic orthostatic hypovolemia in these subjects was unanticipated but will probably alter how we understand and treat these patients in the future.

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Jacob, G., Atkinson, D., Shannon, J.R., Black, B.K., Furlan, R., and Robertson, D. (abstract) Evidence of cerebral blood flow abnoralities in idiopathic hyperadrenergic state. Circulation, 94 (Suppl. 1):I-545 (1996).

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Mechanisms of Antiarrhythmic Drug Action

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Funding:

Project Identification: 199-08-17-72
Solicitation:
Initial Funding Date: 10/94
Expiration: 7/97
FY 1996 Funding: $
Students Funded Under Research: 2
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

The overall goal of this project is to determine the mechanisms underlying the highly variable effect of antiarrhythmic drug therapy. In some patients, treatment with antiarrhythmic drugs can be lifesaving, whereas in others the same drugs may be ineffective or even provoke life-threatening arrhythmias. Clinical and *in vitro* evidence strongly suggests that one factor which modulates response to antiarrhythmic drug therapy is autonomic tone, and projects investigating the effects of activation of intracellular signalling mechanisms on ion channel function at the molecular and cellular levels are in place in this program.

This proposal has two major goals: first, to develop models of sympathetic inhibition relevant to the study of microgravity and, second, to evaluate the effects of such sympathetic inhibition on response to antiarrhythmic drug therapy. Despite the obvious stresses accompanying space travel, plasma norepinephrine is remarkably decreased in astronauts. Thus, the first aim of this proposal will be to develop models of the reduction of sympathetic activity produced by simulated microgravity. Three approaches, the norepinephrine release inhibitor guanadrel, the central a2 agonist clonidine, and prolonged bedrest will be assessed. State-of-the-science techniques to assess sympathetic function, including measurements of norepinephrine spillover and clearance, spectral analysis of heart rate, and direct measurement of sympathetic nerve traffic with microneurography, will be used. Preliminary studies strongly suggest that basal QT interval and QT prolongation induced by drugs such as quinidine are influenced by sympathetic activity. Therefore, the second aim of this proposal is to use the clinical models of reduced sympathetic activity to test the hypothesis that sympathetic inhibition will exaggerate the QT prolonging effects of quinidine in human subjects.

This 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 bedrest studies. In addition, this regimen seems to be well-tolerated by the subjects.

Currently we are performing a modified bedrest 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 bedrest 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 and a raised upright plasma norepinephrine (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 a new environment. Developing Earth-based models for changes produced by space travel will allow us to be better prepared to design countermeasures.

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Architecture and Mechanical Function in Bone with Recovery from Disuse Osteoporosis

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Funding:

Project Identification: 199-26-17-19
Solicitation: 95-OLMSA-01
Initial Funding Date: 03/96
Expiration: 02/97
FY 1996 Funding: $ 198,980
Students Funded Under Research: 3

Task Description:

Disuse results in a loss of bone mass estimated to be an order of magnitude greater than that in any other metabolic disorder of bone. Reversal of an extant osteoporosis is thought to result in recovery of bone mass but not the restoration of microarchitecture or the replacement of lost trabecular elements. Specifically, it is thought that restoration of bone mass after osteoporosis occurs through compensatory thickening of remaining trabecular elements; restoration of trabecular bone microarchitecture (lost trabeculae, interconnectedness of trabecular elements) is thought not to occur. However, there is very little direct information available to support or refute these assertions. The functional-mechanical consequences of having fewer thick trabeculae versus smaller, more numerous, interconnected trabecular elements have been the subject of extensive discussion, but again there is very little direct data on these structure-function relationships. Understanding the recovery potentials of the osteoporotic skeleton architecturally, mechanically, and biologically, has considerable clinical and functional significance.

Our recent studies show that during disuse-induced bone loss in the canine skeleton, there is a discrete, temporal separation of thinning of trabeculae from the later perforation and complete loss of trabecular elements. In the proposed experiments, we will take advantage of this sequence of bone changes in the development of disuse-induced bone loss to establish different architectural baseline points from which recovery of structure and mechanical function with reloading can be examined. We will use a cast immobilization of the canine forelimb to establish baseline points for remobilization. After remobilization, bone microarchitecture will be nondestructively evaluated using microcomputed tomography, and then tested mechanically to determine the mechanical integrity of bone after recovery from disuse.

Experimental studies were initiated in September 1996, and studies are progressing according to schedule.

Osteoporosis is a loss of bone which accompanies a number of skeletal processes, most notably the onset of menopause, decreased mechanical usage, and aging. In these instances, bone losses are characterized as deficits of bone volume or increases in tissue void space (e.g. thinning of bone cortices, trabecular rarefaction); they are considered osteoporoses. Qualitative changes in the bone matrix, such as changes in the relative mineral or organic fractions, are not typically implicated in the pathophysiology of osteoporosis.

Bone loss secondary to decreased mechanical usage follows space-flight, long-term immobilization, and prolonged bed rest, and may also be implicated in the local bone loss processes which accompany implant loosening. Significantly, disuse osteoporosis results in a loss of bone from all skeletal envelopes that is estimated to be an order of magnitude greater than that in any other metabolic disorder which affects bone. It is widely held that the osteoporosis resulting from decreased mechanical usage is reversible - with restoration of normal mechanical usage bone architecture and mass return to normal. However, there is substantial evidence which suggests that reversibility may not be possible or is at least markedly limited. The biological and architectural bases which allow complete versus limited reversal of disuse osteoporosis may have significant implications to the treatment and reversibility of other osteoporoses.

Hypodynamic states, such as space flight and immobilization, result in a loss of bone mass as well as dramatic changes in bone architecture. Recent studies in our laboratories and by others show that in trabecular bone these changes are caused by a combination of both thinning of trabeculae and by perforation of trabecular plates, resulting in complete loss of trabecular elements. Reversal of an osteoporsis is thought to result in recovery of bone mass but not the restoration of microarchitecture or the replacement of lost trabecular elements. Specifically, it is thought that restoration of bone mass after osteoporosis occurs through compensatory thickening of remaining trabecular elements. Restoration of trabecular bone microarchitecture (lost trabeculae, interconnectedness of trabecular elements) is thought not to occur. Surprisingly, however, there is very little direct information available to support or refute these assertions.

The functional-mechanical consequences of having fewer thick trabeculae versus smaller, more numerous, interconnected trabecular elements have been the subject of extensive discussion, but again there is very little direct data on these structure-funtion relationships. Understanding the recovery potentials of the osteoporotic skeleton, both biologically and architecturally, has considerable clinical and functional signficance.

Vestibular Contributions to Post-Spaceflight Orthostatic Intolerance: A Parabolic Flight Model

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Funding:

Project Identification: 199-16-11-56
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $68,000
Students Funded Under Research: 0

Responsible NASA Center: JSC

Task Description:

Astronauts experiencing greater degrees of motion sickness during and/or after space flight often have poorer postflight orthostatic tolerance than their nonmotion sick (or less motion sick) crewmates, even when fluid losses attributable to nausea or emesis are minimal or reversed in the motion sick individuals. This observation is intriguing because: (1) data from multiple invasive animal studies clearly demonstrate that neuroanatomic connections and neurophysiologic relationships exist between vestibular and cardiovascular control areas in the central autonomic nervous system; and (2) data from noninvasive *human* studies also suggest that significant cardiovascular changes can occur during vestibular stimulation, particularly when motion sickness is induced.

In this study, seated parabolic flight on NASA's KC-135 aircraft 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-cardiac baroreflex studies, heart rate variability tests, and head-up tilt) *after the onset of motion sickness*s are 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.

Sixteen unmedicated test subjects have completed our 40-parabola KC-135 protocol. Of these sixteen subjects, ten were susceptible to varying degrees of motion sickness and six were not. Of the ten susceptible subjects, six had emesis during and/or after parabolic flight and four did not. Of the six subjects who had emesis, three were "very susceptible" to motion sickness (i.e., repeated bouts of emesis throughout most of the flight) and three were "moderately susceptible" (i.e., only one or two bouts of emesis toward the end of flight). "Mildly susceptible" subjects were the four subjects who had prodromal symptoms but who did not vomit at any time during or after flight. These subjects were differentiated from the six completely resistant subjects, whose Graybiel motion sickness scores never exceeded 0-1 at any point during or after parabolic flight.

Baseline salivary amylase levels were measured in all sixteen subjects (preflight). Mean levels for each of the motion sickness susceptibility groups outlined above were as follows: (1) "Very susceptible" group (n=3): 337,433 U/L; (2) "Moderately susceptible" group (n=3): 161,000 U/L; (3) "Mildly susceptible" group (n=4) 105,275 U/L; and (4) "Resistant" group (n=6): 114,167 U/L. Thus, baseline salivary amylase levels are higher, on average, in individuals most susceptible to parabolic flight-induced motion sickness. These preliminary findings are similar to the findings of Gordon et al. for subjects experiencing either a seasickness stimulus or a cross-coupled (Coriolis) stimulus.

Of the sixteen subjects studied, two females were not able to complete 30-min tilt tests *preflight* due to typical vasovagal reactions. The other 14 subjects (ten males, four females) finished 30-min preflight tilt tests without symptoms.

Postflight, five of the sixteen subjects were not able to complete 30-min tilt tests, and three additional subjects experienced symptoms while managing to finish. The remaining eight subjects were asymptomatic, finishing their postflight tilt tests uneventfully.

Of the five subjects with frank orthostatic intolerance postflight, three subgroups were identified based upon the pattern of intolerance observed: (1) tilt-intolerant group one, consisting of two females who had hypotensive vasovagal reactions. The postflight vasovagal reaction of one of these females was similar to (but relatively earlier than) the vasovagal reaction she experienced preflight. The other female experiencing a vasovagal reaction postflight did not experience a vasovagal reaction preflight; (2) tilt-intolerant group two, consisting of one male subject who developed significant, symptomatic orthostatic tachycardia postflight (clinically analogous to a patient with the "postural orthostatic tachycardia syndrome," or "POTS," and accompanied by mild orthostatic hypertension rather than hypotension); and, (3) tilt-intolerant group 3, consisting of two males who had what we would term "prostration" - i.e., extreme discomfort in the upright position necessitating tilt-back, sometimes not accompanied by a clear change in vital signs, but often preceded by falls in middle cerebral arterial blood flow velocity as measured by transcranial Doppler [Note that the "prostration" type of intolerance has also been described by Buckey et al. for two astronauts post-spaceflight (Orthostatic intolerance after spaceflight. *J. Appl. Physiol.* 81(1):7-18, 1996), although transcranial Doppler measurements were not performed in that investigation.]

Interestingly, the two subjects in the "prostration" group above both had emesis during parabolic flight, whereas the "POTS-like" subject in tilt-intolerant group two did not. Of the two females with postflight vasovagal syncope (i.e., "tilt-intolerant group one" above), one had emesis during parabolic flight while one did not.

Of the three subjects who had symptoms during postflight tilt testing but who managed to finish the 30-min test, one (a motion-sickness resistant female) had symptoms (lightheadedness) only toward the end of the test, when her hemodynamic pattern began to resemble that of the male "POTS-like" subject in tilt-intolerant group two above. The other two symptomatic finishers (both vomiters during parabolic flight) verbalized but tolerated intermittent symptoms similar to those expressed by the frankly intolerant "prostration" group above (i.e., nausea and lightheadedness sometimes unaccompanied by changes in vital signs). The postflight tilt test of one of these latter "symptomatic finishers" was particularly instructive. This subject refused the relief of tilt-back despite occasional episodes of emesis in the upright position. Her upright episodes of emesis were preceded by nausea, which as it progressed, became accompanied by the following hemodynamic changes: (1) relative

tachycardia; (2) relative hypotension; (3) falls in stroke volume and total peripheral resistance; and (4) falls in middle cerebral arterial blood flow velocity as measured by transcranial Doppler. Repeatedly, just prior to the time when medical monitoring would dictate that her tilt test be terminated on hemodynamic grounds, this subject's nausea would reach a crescendo in an episode of emesis, followed (after some Valsalva-like activity during retching) by a complete *resetting* of her vital signs and a return to stable, upright, pre-nausea hemodynamics. These findings suggest that signals from the vestibular receptors (which are necessary for motion sickness induction) may contribute to the regulation of upright hemodynamics in man, possibly mediated via brainstem areas that influence both cardiovascular *and visceral* responses to nauseogenic stimuli. Findings also suggest that the physical act of vomiting during severe motion sickness may actually be hemodynamically *protective,* since the emetic reflex appears to ameliorate an impending exhaustion of the sympathetic nervous system associated with ever-increasing levels of nausea.

We have tentatively concluded that the following factors may be associated with an increased risk for deficits in orthostatic tolerance post-parabolic flight compared to pre-parabolic flight:

1. *Female gender*: Four of the six female subjects (66%) had lessened orthostatic tolerance postflight compared to preflight, in contrast to three of ten males (30%). Of the two female subjects who did not experience a pre- to postflight deterioration in tilt-test performance, one was completely stable and asymptomatic during *both* the pre- and postflight tilt tests, while the other had a vasovagal reaction preflight but not postflight (postflight, this final female subject was a "symptomatic finisher of the prostration type").

2. *Motion sickness:* Four of the six subjects who vomited during parabolic flight (66%) experienced a pre- to postflight deterioration in tilt-test performance in contrast to three of ten subjects (30%) who did not vomit. Of the two vomiters who did not experience a pre- to postflight deterioration in tilt-test performance, one (a male "moderately susceptible" to motion sickness) was completely free of motion sickness symptoms by the time of his postflight tilt-test. The other (a female "very susceptible" to motion sickness) was the same subject mentioned at the end of the "female gender" risk factor section above, who had a vasovagal reaction to tilt *preflight* but who finished tilt (symptomatically) postflight. Of the ten subjects who had *any degree* of motion sickness during flight, five (50%) had a deterioration in tilt-test performance postflight, compared to two of six subjects (33%) in the group completely resistant to motion sickness. Overall, these findings suggest that while motion sickness and emesis-related fluid loss commonly accompany post-parabolic flight orthostatic intolerance, these conditions are not sufficient to explain intolerance in certain individuals.

3. *Higher than average basal salivary amylase level:* The mean basal salivary amylase level for the entire group (n=16) was 162,588 U/L. The mean basal salivary amylase level for the seven subjects who experienced a pre- to postflight deterioration in tilt-test performance was 227,529 U/L. The mean basal salivary amylase level for the nine subjects who did *not* experience a pre- to postflight deterioration in tilt-test performance was 112,078 U/L. Thus, like the amylase data presented earlier with respect to motion sickness susceptibility, higher mean basal salivary amylase levels were also found in individuals most susceptible to postflight deteriorations in tilt test performance.

4. *First time flyer:* Of the seven subjects who experienced a pre- to postflight deterioration in tilt-test performance, four (57%) had no prior parabolic or acrobatic flight experience. Of the nine subjects who did not experience a pre- to postflight deterioration in tilt-test performance, only two (22%) had no prior parabolic or acrobatic flight experience.

More detailed statistical analyses of the data are in progress.

Statistical analyses of data related to the effects of motion sickness on Valsalva responses, carotid-cardiac baroreflex responses, and heart rate variability are also in progress. Power spectral density analyses of the R-R intervals of some subjects (both motion sick and non-motion sick) have been performed. R-R interval data were collected during controlled frequency breathing prior to takeoff and immediately after landing in both the supine and seated positions. In subjects with recent emesis, supine measurements of total power (0.0-0.3 Hz), high

frequency power (0.2-0.3 Hz), low frequency power (0.05-0.15 Hz), and very-low frequency power (<0.05 Hz) have all tended to increase postflight (i.e., immediately after emetic episodes) versus preflight. However, the rises in high frequency power after emesis are proportionately much greater than the rises in low frequency power, suggesting a relative increase in parasympathetic modulation. In subjects insusceptible to emesis, on the other hand, *declines* in total power, high frequency power, and very low frequency power are more common postflight (vs preflight), accompanied by *increases* in low frequency power. These increases in low frequency power and decreases in high frequency power result in a higher "low-to-high frequency ratio" postflight in emesis-resistant subjects, suggesting a relative increase in *sympathetic* modulation. Further analyses will be helpful in determining the statistical consistency of these trends.

Data pertinent to the effects of acute microgravity and hypergravity on autonomic cardiovascular responses to the Valsalva maneuver have been analyzed, and were presented at the 12th Man in Space Symposium in Washington, D.C., June 8-13, 1996. In brief, seated late-phase II mean arterial pressure (MAP) rises are significantly attenuated in acute microgravity compared to seated late-phase II MAP rises in either acute hypergravity ($p<0.01$) or normogravity ($p<0.01$). Seated phase IV MAP rises are likewise attenuated in acute microgravity [versus acute hypergravity ($p<0.05$), but not versus normogravity]. Seated *early*-phase II Valsalva MAP falls (or troughs) are not significantly changed across any of the gravitational conditions, in contrast to the lower early-phase II falls/troughs seen after transitions from the supine to the seated position in normal gravity ($p<0.05$).

Earth benefits of this overall research include an enhancement of our understanding of the role that the vestibular apparatus plays in regulating the human cardiovascular system, particularly as it relates to orthostatic tolerance. Information gained from this research may prove useful for the development of new therapeutics for both syncope and motion sickness.

FY96 Publications, Presentations, and Other Accomplishments:

Ertl, A.C., Schlegel, T.T., Mitsky, V.P., Snell, M.P., and Robertson, R.M. (abstract) The effect of fluorocortisone and salt loading on orthostatic tolerance following 7 days of six-degree head down bed rest. Clin. Auton. Res., 5, 326 (1995).

Effects of Hindlimb Suspension on Skeletal Muscle Growth

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Funding:

Project Identification: 199-40-27-19
Initial Funding Date: 4/94
FY 1996 Funding: $83,634

Solicitation: 01-13-94/GB
Expiration: 3/97
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 three days after HS when all mitotic divisions are abolished in the soleus muscle. After a complete cessation of divisions that lasts through five days, a low level of divisions resumes. Proliferations appear to remain suppressed for as long as the limb is non-weightbearing in muscle such as the soleus although in non-antigravity muscles such as the extensor digitorum longus (EDL) proliferations may return to near-control levels after extended periods. It is not known whether satellite cell proliferations will exhibit any compensatory increase when weightbearing (WB) is resumed after a period of HS. In this 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.

Over the past year we have completed a study on the effects of weightlessness on regeneration. The effects of hindlimb suspension (HS) on muscle regeneration were examined in soleus muscles of adult rats induced to regenerate by the injection of the myotoxic venom Notexin. The regeneration that occurs following injection can be operationally divided into two phases: (1) a period of high cellular proliferation when myoblasts attain high numbers prior to their fusion into myotubes, and (2) growth of the formed myofibers. We determined that after 10 days of regeneration, regenerates of HS animals were significantly smaller than regenerates of weightbearing animals. The 10-day period encompassed the entire phase of myoblast proliferation and a portion of the myotube/myofiber growth phase. Thus, the reduction in the size of regenerates could result from an the influence of hindlimb suspension on one or both phases. The initial phase of regeneration, which is heavily dependent upon activation and mitoses of satellite cells, was examined with markers for mitotic activity. Using immunocytochemistry after BrdU injections and image analysis, we found no significant difference in the mitotic activity of myogenic and non-myogenic cells in the regenerating HS muscles compared to regenerating weightbearing control muscles. Labeling in isolated fiber segments that contained only myogenic cells (satellite cells and myonuclei) also exhibited no significant difference from weightbearing controls. The results of these studies demonstrated that the weightless environment altered the course of regeneration to produce smaller regenerates. Surprisingly, the initial phase of regeneration (dependent on myoblast mitotic activity) was unaltered even though in contralateral undamaged muscles, satellite cells were mitotically quiescent, as seen in our previous studies. Rather, after the fibers were formed, development was retarded in a manner similar to what we have observed in growing muscles. We concluded that the weightless environment has no direct effect on satellite cells, but exerts an influence on satellite cell mitotic behavior through a myofiber pathway. We have not examined muscle regenerated under conditions of HS to determine whether there is a compensation at longer periods of regeneration or after weightbearing is reinitiated. The results of these studies, together with the reduced growth of immature muscles during HS and in irradiated muscles, support our working hypothesis that there are no compensatory changes in the satellite cell/myofiber axis to enhance development after myonuclear accretion is reduced during the growth period. A manuscript related to these studies is currently under review.

In a second study we examined whether the satellite cell population in a growing muscle could be altered by a period of weightlessness. The feasibility of detecting a change in the satellite cell population was tested by examining the distribution of colony sizes from the soleus muscles from HS and control animals (n=3, each group). Each of the three HS animals exhibited a similar response to HS. There was a decrease in the cells that give rise to small colonies and a corresponding relative increase in the cells giving rise to large colonies. We have not yet been able to statistically analyze the results, but by inspection, they suggest that changes can be detected in the distribution of colony sizes as a result of HS. At 14 days there appears to be a relative decrease in the small colonies (producer cells) and an increase in the relative number of large colonies (progenitor cells). These preliminary experiments suggest that the distribution of cells within the satellite population giving rise to colonies of different sizes is altered by HS.

Finally, in preliminary experiments we asked whether apoptosis was a component of skeletal muscle atrophy. Apoptosis, as used in skeletal muscle would mean the loss of myonuclei from the fiber without death of the fiber. For these experiments we chose a denervation paradigm because of the severe atrophy that occurs in a very short period. Soleus muscles of mature rats were denervated by sectioning the sciatic nerve. We found there was a 60% decrease in mass of the denervated soleus muscle within one week of denervation and an additional 40% atrophy over the next two weeks. We examined the contribution of nuclear loss to muscle atrophy using the TUNEL method (TdT-mediated dUTP Nick-End Labeling), that incorporates flourescein labeled dUTP onto the ends of the fragmented DNA undergoing degeneration (apoptosis). All myofiber nuclei were fluorescein labeled in DNase-treated positive controls. We found a low level (0.1-0.3%) of apoptosis in the denervated muscles one week after nerve section. The incidence of labeled nuclei was higher in the oxidative soleus muscle than the glycolytic extensor digitorum longus. There were no labeled nuclei in the control muscles. DNA gel electrophoresis results corroborated the morphological findings. Light smears of DNA, indicating apoptosis (Schwartsman and Cidlowski, 1994) were present in the denervated soleus lanes but not control lanes. These preliminary morphological and gel electrophoresis studies indicate that apoptosis is associated with atrophy of mature muscles.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Ermini, E.B., Ford, C.N., and Schultz, E. (abstract) The rat as a model for assessing the results of botulinum toxin on laryngeal muscles. Res. in Otolaryngol., 20 (1995).

Inagi, K., Ford, C.N., Schultz, E., Rodriquez, A.A., and Pasic, R.R. (abstract) Physiologic assessment of botulinum toxin effect in the rat larynx. Otolaryngology-Head Neck Surg. 115:117 (1996).

Inagi, K., Schultz, E., and Ford, C.N. (abstract) An anatomical study of the rat larynx. Otolarngology-Head and Neck Surgery 113:175 (1995).

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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 1996 Funding: $ 128,921
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 one 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 two, pulmonary circulation will be added, and in year three 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 zero-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.

An hydraulic model of the human systemic circulation was flown aboard the NASA KC-135 to study the effects of gravity on the cardiovascular system. The model incorporated an artificial heart, a caudal venous pool and three vascular sections to allow investigation of fluid shifting in the vasculature as well as changes in cardiac performance due to gravity. An extensive computer model, which incorporated over 1000 elements (lumped resistance, compliance and inertance), was used to choose values of the elements in the regional mock circulation units most closely approximating the human system. The aortic input impedance of the numerical model were first compared to human data. Values of compliance and artery segment radius (and thus resistance and inductance) were systematically adjusted until an acceptable match for the human physiologic responses were achieved. The arterial tree was then divided into body sections according to their position relative to the heart - a cranial region for the shoulders, arms, hands, neck and head, a caudal section for the legs and a central section for the major organs (kidneys, liver, lungs, and intestines). The impedance spectra of each section were used as targets for the development of simplified circuits to be incorporated into the hydraulic model. An iterative procedure was used to find the element values providing the best fit to the steady flow resistance and the first harmonic impedance modulus and phase. Each vascular section had four elements - arterial resistor, arterial compliance, peripheral resistor and peripheral compliance. A small amount of inertance was also introduced in connecting tubing. This arrangement of elements produced very physiologic pressure and flow wave forms. The resistors were made from a porous plastic sheet over which a motorized plate slid for adjustment of the cross sectional area available for flow, and therefore the resistance value. Each compliance unit incorporated a coil spring-loaded piston moving inside a cylinder sealed with an elastomeric diaphragm [Woodruff, *et al.* 1995]. The compliance values were adjustable by changing the spring. The model of the left ventricle consisted of a flexing, polymer sac inside a pressurization chamber. Ultrasonic crystals for the measurement of ventricular diameter were incorporated into the walls of the ventricular sac 180° apart, half way between the base and apex of the ventricle. An artificial atrium made of a flexible polymer sphere was connected upstream of the inflow valve. This combination of dimensions and pulsatile function approximates the anatomy, endocardial wall movement and pressure-volume relationship of the natural left ventricle. The peripheral venous pool (PVP) consisted of a flaccid, polymer sac inside a rigid cylindrical chamber. The nonlinear response of the venous pool was similar to that reported for natural veins. The experiment was monitored with four flow probes, ten pressure transducers, a pair of ultrasonic crystals, six displacement transducers and one accelerometer. Heart function curves depicting cardiac output versus left atrial pressure were generated using data collected from the hydraulic model in launch, supine and upright postures aboard the KC-135. In all postures, there was a measurable shift to the right of the heart function curves from 2-G to 0-G. In all postures, as filling pressure increased in 0-G, cardiac stroke volume also increased. This is a direct result of a shift toward the atrium of fluid during entry into 0-G. The left ventricle was subjected to a 5 mmHg vacuum during diastole to simulate the unloading effects of acceleration on the chest wall upon entry into 0-G for the upright posture. A leftward shift of the heart function curve with an increase in cardiac output in the presence of reduced filling pressure was observed. This observation gives additional support to the hypothesis that this paradoxical increase in cardiac size and stroke volume, as reported from SLS-1 and SLS-2 missions, is caused by an increase in cardiac transmural pressure via a reduction in the intraplural pressure when the acceleration-dependent loading of the chest wall is removed. Regional circulating fluid shifts were measured for the first time in the hydraulic model aboard the NASA KC-135 and on the ground. The regional flows and the aortic pressure were adjusted to physiologic values in level flight (1-G) and then the model was observed, with no further adjustments or control, throughout the 0-G and 2-G parabolic flight of the KC-135. The fluid shifts, therefore, represented the combined effects of changing hydrostatic pressure gradient on both the fluid circulating loop and the heart itself. It has been previously documented that the presence of a hydrostatic pressure difference within the ventricle increases diastolic filling and cardiac output [Pantalos, *et al.* 1996]. This effect tends to increase cardiac output and aortic pressure, as well as all three regional arterial pressures. The hydrostatic effect on the fluid column of the hydraulic model is to increase pressure below the hydrostatic indifference level (HIL) and decrease pressure above the HIL with increasing gravitational acceleration. The location of the HIL depends on the vertical distribution of compliance in the model, with the HIL moving toward increasing compliance and away from decreasing compliance. In the hydraulic model, compliance changes in two ways. First, the otherwise linear compliance spring-loaded bellows may top out at high pressure, partially simulating the full inflation of the regional vasculature. The bellows may also bottom out at low pressure, simulating the collapse of the regional vasculature. Second, the PVP

chamber is designed to be nonlinear to mimic the collapse and inflation characteristics of the veins. The PVP represents a large fraction of the fluid volume available for shifting, about ten times the volume of an individual spring-loaded compliance unit. In addition, the cardiac output is strongly dependent on the atrial pressure, which is influenced during changes in gravitational acceleration by the location of the HIL. Higher atrial pressure, of course, leads to increased diastolic filling and increased cardiac output. The interaction of all these effects caused the regional fluid shifts witnessed during the parabolic flights. For the supine posture, the hydrostatic effect on the circulation is minimal, however a small effect due to the anterior-posterior hydrostatic pressure difference within the ventricle has previously been documented [Pantalos, *et al.* 1996]. For the launch posture, a moderate shift of fluid occurred from the PVP to the system between 0-G and 2-G, with the PVP essentially emptying into the system with the increased gravitational pull. Regional fluid shifts were most evident in the upright posture. As expected, fluid shifted away from cranial compliance elements and toward caudal compliance elements with increasing G level. The magnitude of the shift depended on the systemic hydration level with larger shifts corresponding to greater circulating fluid volume. Venous volume changes were larger than arterial volume changes, due to the larger compliance values on the venous side. Due to its caudal position, the PVP volume also increased substantially between 0-G and 2-G. In the upright position, the PVP operated in a range of high pressure and low compliance, simulating fully inflated vessels. Curiously, the central venous and arterial compliance units demonstrated opposite shifts. It should be remembered that the flow rate through the system also changed simultaneously with the fluid shifts. The change in pressure between 0-G and 2-G is substantial in both the cranial and caudal units, making their responses dominated by the hydrostatic pressure change, however the pressure change is not so strong in the central units. The interaction of these responses is still being investigated.

Hypotension and tachycardia are severe for many astronauts. Approximately half cannot tolerate a 10-minute stand test immediately after landing. Post-flight orthostatic intolerance first appeared after the fourth manned Mercury flight of only 34 hours and has occurred after flights of just nine hours. Most nonastronauts have experienced orthostatic intolerance at one time or another and for some people the effects are chronic and debilitating. While long-term adaptations to microgravity may contribute to reduced tolerance, it is clear from the above results and from patients on Earth that short to intermediate-term effects must play an important role. Increased leg compliance, increased capillary permeability, deteriorated baroreceptor response, and hypovolemia are some of the causes that have been forwarded. The partial success of pre-landing ingestion of saline in preventing orthostatic intolerance indicates that hypovolemia is at least partially responsible, however, these results do not preclude the contributory effects of other factors. This project focuses on the effect of changes in hydrostatic pressure on the cardiovascular system, an effect that is present not only in launch and landing for astronauts, but also during changes in posture for people on Earth. Further study of this mechanism may lead to more effective countermeasures for all sufferers of orthostatic intolerance.

FY96 Publications, Presentations, and Other Accomplishments:

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Integration of Multidisciplinary Sensory Data [Human Brain Project]

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Funding:

Project Identification: n/a

Solicitation:

Initial Funding Date: 9/95

Expiration: 8/96

FY 1996 Funding: $190,000

Students Funded Under Research: 4

Joint Agency Participation: NIH and Human

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 this PI make this integrative approach to the system possible, but progress is blocked by the lack of enabling technologies for effectively interconnecting the 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. 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 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; and 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.

A. Olfactory Receptor Database (ORDB) (Healy, Skoufos, Singer, Smith, Nadkarni, Miller, Shepherd)

This database went on-line last summer, and has continued under development to meet user needs. These needs were in several areas. First was the need to respond to user requests for modifications to improve ease of use across a range of different users. Since different users have different preferences, this has required considerable effort. The brisk usage also has required continual updating of the files of newly deposited sequences. The number of sequences has grown (now over 200 unpublished sequences and over 200 published sequences). A new person, Dr. Emmanuel Skoufos, was hired in October to join with Dr. Healy in handling the increased load. As a trained informatics specialist, Dr. Healy continues to be responsible for the development of the database itself, while Dr. Skoufos, a molecular biologist interested in informatics, is training in database techniques with Dr. Healy and is responsible for the interactions with scientist users. Part of our pilot function has been to institute procedures for monitoring the use of the database. These show that use of ORDB is increasing gradually, and is now approximately 30 hits a month by real users, after subtracting hits by search engines and development here at Yale.

Dr. Skoufos is also responsible for carrying out further research on the database using digital tools specifically developed for this purpose. The first project has been to bring some order in the classification of the receptors by different laboratories by developing a rational system of nomenclature to classify and organize this vast gene family. A manuscript on the ORDB (Healy et al, 1997) is in press; a manuscript on olfactory receptor nomenclature is under review (Skoufos et al, submitted).

B. Neuron Database (NeuronDB) (Mirsky, Nadkarni, Zufall, Leinders-Zufall, Chen, Miller, Shepherd)

A pilot database of neuron properties underlying circuit functions was constructed last year in order to lay the foundation for a true database. This enabled us to explore the problems of organizing these complex properties across the full range of types of neurons that are present in the nervous system. That phase was completed and construction was started last summer on a combined relational/object oriented database using Illustra. Jason Mirsky has been working full time on this with Dr. Shepherd, with valuable consultation with Dr. Nadkarni and Dr. Miller.

This database is called Neuron Database (NeuronDB) (http://senselab.yale.med.edu/neurondb). It is aimed at enhancing the ability of neuroscientists to quickly integrate information about the neuronal properties of specific neuronal types into computational models of these types, and to immediately assess a given property, or all of the properties, of a given type by comparison with other types. A searchable database provides a unique tool for accomplishing both of these aims in a rapid, efficient, and accurate manner. The three main types of properties we have initially concentrated on are neurotransmitters, neurotransmitter receptors, and active membrane properties. We are starting with the three main types of of projection neurons that make up the olfactory pathway: olfactory receptor neuron, mitral/tufted cell, and olfactory cortical pyramidal neuron. We are also including the projection neurons for several other regions of the brain, as in the pilot, in order to be sure that the tools are sufficiently flexible to handle the extraordinary complexity of neuronal types in the nervous system. A key in dealing with this complexity has been to identify canonical forms of each neuronal type, with standardized structures applied across different types. This has enabled the database to be searchable for different

combinations of specific properties within specific compartments or neuronal structures, a function that to our knowledge is not provided by any other database of these neuronal properties.

We are at present finishing the first phase of development of NeuronDB. The database consists of three parts: a database server and a web server, and CGI (Common Gateway Interface) programs that link them together. It uses Illustra software, which combines the power of object oriented programming without losing the flexibility of the relational database environment. Two manuscripts are in preparation, one on the details of database construction for a journal in the field of informatics, and one on specific applications in neuroscience.

C. Model Database (ModelDB) (Hines, Chen, Mirsky, Shepherd, Nadkarni, Miller)

Last year we carried out pilot studies to build digitally-based tools for generating neuronal models and for analysing them; this work was principally by Dr. Bret Peterson. This work was put on hold after he left for a job in the commercial sector in March 1996. However, as also documented in our progress report, we were interacting increasingly with Dr. Michael Hines in our effort toward computational modelling. This effort received a big boost when Dr. Hines, the developer of the neuronal modeling program NEURON, accepted our invitation to join the research group last fall. Since then, we have been collaborating closely with him on developing tools for enhancing the construction of neuronal models. Previously, Dr. Peterson had an interest in running the models over the Web using Java, which had the disadvantage of being very slow (minutes); with Dr. Hines, we are concentrating on running NEURON directly on our own workstations, where it is very fast (seconds). This change in direction means that we have the opportunity to build our ModelDB in close collaboraton with the person who created and maintains one of the two main neuronal modeling programs. A collaboration between Dr. Hines and Dr. Chen will model the results. Dr. Chen is obtaining in experiments with a visitor, Dr. Jens Midtgaard from Copenhagen, using double patch recordings from the mitral cell dendrites. In addition, Jason Mirsky is working with Dr. Hines to develop the interface between NeuronDB and ModelDB, so that properties resident in NeuronDB can be inputted directly into ModelDB.

D. Ongoing Collaborations

Beginning last summer we initiated a collaborative project with Dr. Charles Greer in Neurosurgery and Dr. Robert Shulman in Molecular Biophysics and Biochemistry using fMRI to image odor-induced activity in the rat olfactory bulb under high Tesla (4.7T) at the laminar and modular level. This has opened up a new method for mapping odor induced activity in the olfactory bulb. In order to construct these maps we are interacting with Dr. David van Essen, another member of the Human Brain Project, to adapt his mapping softwared CARET, used for mapping large cortical regions, to the small dimensions of the olfactory bulb.

E. Changes of Direction

One change of direction continues to be ORDB, which was not envisioned in the original grant application. A second new change in direction is associated with Dr. Hines joining the group, which gives us a unique opportunity to pursue collaborations combining experimental results and testing with computational models on a close and continuing basis. The development of NeuronDB was not explicit in the original application, but is a natural outgrowth of our focus on integrating multidisciplinary data. The construction of a ModelDB will in turn be a natural outgrowth of this database and the integrative tools it provides.

What questions have been answered.

ORDB is providing a working tool for researchers who are actively cloning and sequencing the very large family of olfactory receptor genes. It is unusual in that it provides tools for storing and searching unpublished sequence data. It is also a direct link between the Human Brain Project and the Human Gene Project.

NeuronDB will be the first attempt to integrate data on different types of membrane properties for specific neuronal types, represented in canonical forms, and to make this data searchable across different neuronal types.

ModelDB, although still largely in the planning stage, will facilitate neuroscientists in taking experimental data for a neuronal type and constructing computational models for testing hypotheses in close correlation with the experimental analysis.

Our new project on fMRI imaging of the olfactory bulb extends this approach to the laminar and cell module levels. It presents the opportunity to apply mapping algorithms developed for large brain regions to these more fundamental levels of neural and glial organization.

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

A. ORDB (Healy, Skoufos, Singer, Smith, Dadkarni, Miller, Shepherd; user group on WWW).

We will continue to develop ORDB for our increasing user group. Drs. Healy and Skoufos will continue to work closely together in improving and extending the structure and functionality of the database, as well as interacting with users to facilitate their use of the database. Toward this end Dr. Skoufos will extend his training in database and informatics methodologies. Dr. Skoufos will also continue work on developing other parts of the ORDB database cluster, including chromosome mapping. This cluster will also include maps of the projection sites of olfactory cell subsets expressing different types of receptors and of the distributions of odor induced activity in the olfactory bulb glomerular layer using our previous methods of 2DG and our new method of fMRI.

B. NeuronDB (Mirsky, Nadkarni, Zufall, Leinders-Zufall, Chen, Miller, Shepherd)

We are aiming to go on-line with this database, replacing our pilot, in the near future. The main goals of this trial period will be to work out the interface with users and with those depositing data, to respond to requests for improving functionality or for new tools, and to carry through the significant task of inputting data for all the compartments of all the neurons. Once we feel reasonably confident about these goals, we will be in a position to add additional types of neurons. In the first instance these will be to extend the database from principal (output) neurons to the intrinsic neurons with different regions. This will provide the eventual basis for constructing circuit models of those regions (see below). In addition, we will extend the database to other types of neurons as requested by users. As a pilot project, we will place special emphasis on security mechanisms as we have already been the subject of several attempts at illegal entry.

C. ModelDB (Hines, Chen, Mirsky, Shepherd, Nadkarni, Miller)

The close collaboration with Dr. Hines on using NEURON to construct computational models of the mitral cell and other olfactory neurons under experimental investigation will continue. In addition, we will continue to develope additions to NEURON that will facilitate archiving and retrievable of trial runs. Finally, we will begin construction of a database of neuronal models (called ModelDB), building on the format of NeuronDB.

D. Continuing Collaborations

fMRI. We will continue our collaboration with Drs. Robert Shulman and Charles Greer on fMRI of the olfactory bulb. We will interact with Dr. David van Essen in applying CARET to mapping this data.

Long-term sensory adaptation. Of possible direct significance to the mission of NASA is recent work in collaboration with Dr. Zufall in the group, that CO acting through cyclic GMP mediates a very long-term type of sensory adaptation in olfactory sensory cells. We will explore a comparison of long-term sensory adaptation in vestibular hair cells compared with olfactory cells. We aim to collaborate with Dr. Muriel Ross and other vestibular organ neuroscientists on whether similar mechanisms could be involved in the sensory adaptation of the vestibular system to zero gravity in space.

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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Funding:

Project Identification: 199-26-17-07
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $114,975
Students Funded Under Research: 37

Task Description:

The overall objectives of this project are to examine the effects of two weeks of bedrest on the sympathetic nerve responses to rhythmic and static forearm exercise. We postulate that sympathetic nerve responses will be increased because lactic acid production and forearm interstitial volume will be increased. We further postulate that forearm handgrip exercise and/or intermittent forearm compression will act as countermeasures to obviate the effects of bedrest.

The grant was initially funded in March 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 bedrest. We were going to use forearm training as a countermeasure to reduce sympathetic nerve responses to forearm exercise. In this report we observed that forearm training attenuates a number of sympathetic neural indices.

With regard to the bedrest studies we have gathered partial sets of data for a number of projects. First, we have gathered resting sympathetic nerve data on 15 individuals before and after bedrest. The data is currently being analyzed. Second, we have gathered MSNA data during handgrip exercise in eight volunteers before and after bedrest. Analysis of this data is not complete. We would like to study an additional group of subjects over the next year to complete this project.

We have also obtained venous effluent metabolite data from five subjects before and after bedrest to examine if bedrest alters the ischemic exercise pressor reflex. To date this data shows no difference in muscle metabolism after bedrest.

In prior reports we demonstrated that limb immobilization reduced peak forearm blood flow (Silber et al. *JAP* 1990; 68(5): 1945-1949). Accordingly, we became interested in determining whether bedrest would reduce peak forearm blood flow. To date we have gathered forearm flow data from 21 volunteers before and after bedrest. Analysis of these data is ongoing. We are currently completing a group of bedrest studies that were started a

month ago (a group of eight subjects completed this most recent bedrest study). Data from this current group of experiments will be analyzed shortly. The next group of six subjects will be studied in July or August of 1997.

The initial reason for performing these experiments was to gain insight into the effects of prolonged space flight on muscle reflexes. We postulated that the increase in interstitial volume and the potential changes in muscle fiber types would lead to a predilection towards heightened sympathetic responses to exercise. Additionally, we speculated that the muscle changes described above could contribute to the heightened sense of forearm fatigue sometimes mentioned by astronauts during EVAs.

It is important to emphasize that bedrest and the accompanying autonomic changes seen are a common accompaniment of many major disease processes. Accordingly, the study of autonomic control after bedrest has major implications for these problems. For example, after a number of illnesses patients are placed at bedrest for a number of days. With the resumption of activity there are a number of important difficulties noted by these patients (e.g., fatigue with limited). The mechanisms for this fatigue are difficult to study in the ill patients. Understanding the ramifications of bedrest have important implications for this issue. Additionally, individuals with cardiovascular disease are often placed at bedrest for a number of days and concurrently receive medication which impairs postural control. An understanding of the vascular and autonomic responses that are due solely to bedrest would also have important implications for our understanding of postural difficulties in patients with severe cardiovascular insults.

FY96 Publications, Presentations, and Other Accomplishments:

Boehmer, J.B. and Sinoway, L.I. Reply to Letter to the Editor. Supplemental oxygen administration and congestive heart failure. J. Am. Coll. Cardiol, 28(5), 1433-1434 (1996).

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Hartz, A.J., Ratner, E.R., Sinoway, L.I., and Bartholomew, M.J. Smoking and idiopathetic congestive cardiomyopathy. Jpn. Hrt. J., 37, 401-407 (1996).

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Potts, J.T., Sinoway, L.I., and Mitchell, J.H. "Afferent mechanisms, medullary sites and efferent sympathetic responses of the exercise pressor reflex" in "Human Kinetics." (in press).

Shoemaker, J.K., Pandey, P., Herr, M.D., Silber, D.H., Yang, Q.X., Smith, M., Gray, K., and Sinoway, L.I. Augmented sympathetic tone alters muscle metabolism during exercise: Lack of metabolic evidence for functional sympatholysis. J. Appl. Physiol., (in press).

Silber, D.H., Sinoway, L.I., Leuenberger, U.A., and Amassian, V.E. Magnetic stimulation of the human motor cortex evokes skin sympathetic nerve activity. J. Appl. Physiol., (in press).

Silber, D.H., Sinoway, L.I., Leuenberger, U.A., and Amassian, V.E. (abstract Magnetic stimulation of the human motor cortex increases skin sympathetic nerve activity (SSNA). FASEB J., 10(3): A8 (1996).

Sinoway, L.I. Neural responses to exercise in humans. Implications for congestive heart failure. Clin. Exp. Pharmacol. Physio., 23, 693-699 (1996).

Sinoway, L.I. Autonomic responses to exercise in heart failure. Department of Medicine - Core Curriculum, The University of Illinois at Chicago (July 31, 1996).

Sinoway, L.I. Clinical observations obtained from direct recordings of sympathetic outflow. Department of Medicine - Grand Rounds, The University of Illinois at Chicago, (August 1, 1996).

Sinoway, L.I. Developing a treatment model for decompensated heart failure: The clinical challenge. Albert Einstein College of Medicine, Bronx, NY (July 9, 1996).

Sinoway, L.I. GCRC presentation. Executive Committee Meeting, The Milton S. Hershey Medical Center, Hershey, PA (July 3, 1996).

Sinoway, L.I. GCRC presentation. Department of Medicine Faculty Meeting, The Milton S. Hershey Medical Center, Hershey, PA (July 23, 1996).

Sinoway, L.I. GCRC presentation. New Faculty, The Milton S. Hershey Medical Center, Hershey, PA (August 21, 1996).

Sinoway, L.I. Reflex responses to exercise. The Milton S. Hershey Medical Center, Hershey, PA (February 1, 1996).

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Sinoway, L.I. Sympathetic nerve discharge in humans. Surgery Research Conference, The Milton S. Hershey Medical Center, Hershey, PA (February 6, 1996).

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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 1996 Funding: $26,452
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.

Six subjects have been studied during one to four days of sleep restriction (4 hours per night). The preliminary data suggest that the most striking findings are: 1) that baseline sympathetic nerve activity, vascular resistance, and blood pressure are not altered by the sleep restriction, 2) sympathetic neural, vascular resistance, and blood pressure responses to stressors, including orthostasis, exercise, and mental stress, are exaggerated after sleep restriction, and 3) orthostatic tolerance may be impaired in selected subjects.

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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Project Identification: 199-18-17-17
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 4/98
FY 1996 Funding: $277,035
Students Funded Under Research: 7

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 approximately 50% of data collection and analyses. We have hired five part-time research assistants (one of them is a visiting trainee from Russia), and we have included in our team two graduate students (one from Brazil) and 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 three-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. We have recently acquired a new, state-of-the-art fully digital polysomnographic ambulatory recorder, and are in the process of upgrading our data collection system to this system.

A difficult and complex task involved subject recruitment (subjects need to spend three months of their time in the lab over a five-month period). Prospective subjects interviews, screening, and pilot recordings have been completed. We have also successfully completed the first five month study, involving three subjects, each following a different schedule for one month at a time. Data analyses are underway and have demonstrated good subject compliance and minimal loss of data due to technical or other problems.

Preliminary findings from this initial pool of subjects suggested that under conditions of extreme sleep reduction, the circadian pacemaker maintains a very stable period of 24 hrs without significant variations in phase, and - contrary to what could be expected — amplitudes of the core body temperature cycle showed no differences whether sleep was taken in one nocturnal sleep episode or divided into multiple naps during the 24 hrs. This preliminary analysis also suggested that the monophasic may not be the best sleep reduction strategy under sustained work. In contrast, the bi-phasic strategy with two naps per day appears to provide the best overall effectiveness. The bi-phasic has the additional advantage of being more practical than the polyphasic approach during real emergency situations.

It is obviously too early for any conclusions given that further analyses are needed on the initial data set and that a second round of experiments will be starting soon.

Results from our previous and ongoing polyphasic sleep studies show that the sleep strategies proposed here may have a significant potential to overcome serious decrements of performance which may be experienced during emergencies in space flight missions. This program combines theoretical and practical interest: it will provide solutions to efficient and safe handling of emergency situations in space, while contributing to our understanding of sleep and alertness regulatory mechanisms. In addition, we will develop tools that may assist in the design of sleep-wake strategies for the growing population of individuals involved in quasi-continuous or irregular work scenarios. The specific aims of our study are: 1) To test the hypothesis that polyphasic sleep allows for dramatic levels of sleep reduction; 2) To test the hypothesis that polyphasic sleep is a practical solution to maintain high levels of efficiency under conditions of quasi-continuous work; 3) To determine the minimum amount of sleep necessary to maintain an acceptable level of performance; 4) To identify the most important factors (such as nap duration and timing, amount of prior wakefulness, nap architecture) that may affect the benefits of naps taken during extended work; 5) To further characterize the architecture of ultrashort sleep and the obligatory components of minimal sleep (e.g., slow-wave sleep, REM sleep); 6) To understand whether phase, period, and amplitude of circadian rhythms are affected by polyphasic sleep schedules. It is also expected that this study will result in significant practical application to NASA, as well as to any other organization dealing with sustained work; 7) Understanding how individuals should be trained to adapt to polyphasic sleep schedules and to develop strategies that would allow rapid transition from monophasic into polyphasic sleep. 8) Defining how individuals vary in their constitutional ability to adapt (or not adapt) to polyphasic sleep; and 9) Determining what are the limits of systematic and prolonged use of polyphasic and ultrashort sleep-wake schedules.

The hypothesis formulated here is 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.

Visual and Vestibular Contributions to Human Heading Estimation (1 year "seed money")

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Project Identification: 199-16-12-37
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/96
FY 1996 Funding: $60,000
Students Funded Under Research: 0

Responsible NASA Center: ARC

Task Description:

The task of navigating through a cluttered environment involves a complex, coordinated sensorimotor process that uses visual, vestibular, proprioceptive, motor-corollary, and cognitive inputs. Determining one's movements (self-motion estimation) and the environmental layout (relative depths) are critical elements of that task. The problem becomes more acute during space flight as astronauts often work in environments where important visual cues may be missing and because microgravity induces changes in both vestibular and oculomotor function. We propose to measure and model visual and vestibular contributions to human self-motion estimation by studying heading and depth discrimination in response to pure visual (flow fields simulating self-motion), pure vestibular (actual translation in darkness), and ultimately combined visual-vestibular stimuli.

Our study of human self-motion perception is currently examining how humans process and integrate visual motion information and how eye movements relate to motion perception. Dr. Thompson at the University of York in the UK and I have previously identified human errors associated with low-contrast motion stimuli (such as motion obscured by fog). In FY 96, we showed that fully contrast-normalized models of human speed perception cannot explain this result. We also found that humans make contrast-induced errors in flicker (temporal frequency) estimation, but that these errors are in the opposite direction as those found for motion. This fact rules out models of human motion perception that rely on flicker perception to derive speed. Dr. Verghese, an NRC postdoctoral associate, and I measured how effectively the information in multiple motion stimuli can be processed simultaneously; we then developed quantitative models that predict the same trends found in the human performance data. We also demonstrated that image segmentation and spatial layout plays a critical role in speed perception. The latter result has important implications for heads-up display design, or the design of any display that uses superimposed imagery, as well as for visual neuroscience in general. Dr. Perrone at the University of Waikato in New Zealand and I showed that heading judgments during simulated motion around a curve show small but systematic errors related to the sharpness of the turn and the simulated speed of the observer. In collaboration with Dr. Mulligan of AFH, Dr. Beutter, another NRC postdoctoral associate, and I 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 design as well as for 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 at the College de France, Dr. Beutter and I showed that humans can accurately track partially occluded objects even when the correct strategy for doing so is not simply to nullify the motion on the retina. This provides a major challenge to current models of human pursuit eye movements. We also found supporting evidence for the view that perceived motion rather than physical stimulus motion drives pursuit.

Our study of human self-motion perception and oculomotor control has numerous significant Earth benefits. First, our model can be used to predict human performance in a variety of navigational tasks from flying to driving. Identifying situations which may lead to human error will provide information critical to engineers designing cockpits, cars, displays, and simulators, and others interested in reducing accidents (instructors, freeway designers, etc.). Second, our psychophysical paradigms will lead to better methods for measuring driver and pilot visual proficiency and for diagnosing subtle pathology in the visual system after an accident/stroke or due to aging. For example, the present method of using visual acuity to test drivers prior to license renewal does not measure the person's true ability to use visual information to navigate. The tasks we have developed to explore human self-motion perception provide a better measure of this ability. Third, our development of new technologies for measuring and analyzing oculomotor data enables the measurement of perception in real time. This new approach could be used to monitor perception in applied and real world settings where the use of standard methodologies is not possible. Fourth, because our models are based on the known physiology and anatomy of primate visual cortex, our results provide fundamental insights into how the primate brain processes and integrates sensorimotor information from multiple modalities (visual, oculomotor, vestibular) to generate a robust perception of self-motion and to guide complex motor behavior.

FY96 Publications, Presentations, and Other Accomplishments:

Beutter, B.R. and Stone, L.S. (abstract) Quantifying the correlation between eye-movement and perceptual responses to moving plaids. Ophthalmology and Visual Sci., Vol 37, p S739 (1996).

Beutter, B.R., Lorenceau, J., and Stone, L.S. (abstract) Visual coherence affects smooth pursuit. Perception Suppl., vol 25, p 5 (1996).

Beutter, B.R., Mulligan, J.B., and Stone, L.S. The barber plaid illusion: Plaid motion is biased by elongated apertures. Vision Research, 36, 3061-3075 (1996).

Chapman, B. and Stone, L.S. Turning a blind eye to cortical receptive fields. Neuron, 16(1), 9-12 (1996).

Stone, L.S. and Perrone, J.A. (abstract) Translation and rotation trade off in human visual heading estimation. Investigative Ophthalmology and Visual Sci. (1996).

Stone, L.S., Lorenceau, J., and Beutter, B.R. (abstract) Smooth pursuit of a partially occluded object. Perception Suppl., Vol 25, P 5 (1996).

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Verghese, P. and Stone, L.S. (abstract) The effect of spatial layout on perceived speed. Investigative Ophthalmology and Visual Sci., Vol 37, p S750 (1996).

Countermeasure for Microgravity-Induced Muscle Atrophy

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Initial Funding Date: 9/94
FY 1996 Funding: $190,000

Solicitation:
Expiration: 9/96
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 IGF-1 system which in turn results in decreased muscle structure protein synthesis.

Using horizontal bedrest 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 second year of this grant have shown by direct methods that bedrest results in decreased muscle mixed protein synthesis. Studies begun in the second year have shown that the decline in amino acid incorporation into muscle protein is associated with a decrease in the level of the mRNA for α-actin, but no change in the amount of β-myosin message. Technical problems in the third year of this proposal have delayed the completion of the analysis of the IGF-1 system (IGF-1, its receptor, and IGF binding proteins expression) in bedrested subjects.

In the remainder of the third year and extending into a few months of a fourth year, the IGF-1 system quantification will be complete. Pharmacological countermeasures with growth hormone treatment during bedrest will begin in the fourth year of this project.

Results of this research include:

1. Quantification of changes in α-actin and β-myosin mRNA in human muscle during bedrest with and without exercise as a counter measure: mRNA for α-actin and β-myosin were measured in thigh skeletal muscle from eight subjects who underwent 14 days of horizontal bedrest. Two of these performed resistance exercise using a horizontal exercise bicycle. Comparison of actin and myosin message levels between these two subject who exercised and the six controls who did not showed no difference. In this study, however, the control subjects did not show any difference before and after bedrest. This is a major distinction from our previous studies in a different group of six subjects. In the former study, there was a significant decline in actin message but no change in myosin. The findings of the current study will be further evaluated and corroborated by performing the same measurements in another six exercising subjects. The fact that the controls did not show a difference in actin message attributable to bedrest gives us concern. We hypothesize that the stringency of the bedrest in the

current study was insufficient because subjects were allowed to use a bedside commode and this may have been enough to alter the muscle biochemical manifestations.

2. Development of techniques for quantification of intramuscular IGF-1 mRNA: The ribonuclease protection assay for human IGF-1 mRNA measurement has been optimized, such that reliable data can be obtained with as little as 15 μg total muscle RNA. This represents about 30 mg of muscle, or about one third of the typical percutaneous muscle biopsy.

3. Development of techniques for measurement of muscle mRNA for the type 1 IGF receptor and for IGF BP3 and IGF BP4: In collaboration with Dr. Randall Urban at UTMB, cDNA generated riboprobes and assays have been refined such that the sensitivities of these ribonuclease protection assays are high enough to allow quantifying the levels of message with less than 5 μg of RNA, thus allowing multiple measurements in addition to the IGF-1 message from a single 100 mg muscle biopsy.

The plans for the remainder of the current year and part of a fourth year include the completion of the IGF-1 system characterization. Following this task, administration of recombinent human growth hormone (rhGH) during bedrest will begin. Response to rhGH will be evaluated in direct analysis of mixed muscle protein synthesis, measurement of mRNA for actin and myosin, measurement of mRNA for IGF-1 and its major binding proteins, and immunoblot quantification of IGF-1 protein in muscle.

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 a potential biochemical control point which is regulated by muscular activity. Pharmacological countermeasures will be tested directly on the muscle protein synthesis system, which will be directly applicable to other muscle atrophy syndromes such as trauma-related immobilization, AIDS wasting, cancer cachexia, and aging.

FY96 Publications, Presentations, and Other Accomplishments:

Ferrando, A.A., Lane, H.W., and Stuart, C.A. Prolonged bedrest decreases skeletal muscle and whole body protein synthesis. Am. J. Physiol., 66270, 976-981E627-E633 (1996).

Williams, W.J., Lee, S.M.C., Fortney, S.M., Stuart, C.A., and Whitson, P.A. (abstract) Cardiovascular responses to LBNP following bed rest: The effects of dietary sodium. FASEB (1996).

Development of an Advanced Video Ocular Measurement System

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Project Identification: 199-70-31-21
Solicitation: 95-OLMSA-01
Initial Funding Date: 3/96
Expiration: 3/97
FY 1996 Funding: $164,181
Students Funded Under Research: 0

Responsible NASA Center: JSC

Task Description:

Video-based tracking systems have come into prominence in several research fields as the preferred eye measurement tool because of the precise multidimensional analysis capabilities they provide. The goal of this project is to build a real time Video Ocular Measurement System (VOMS) based on the prototype VOMS which implemented a new video eye image analysis algorithm for the STS-42 Microgravity Vestibular Investigations (MVI).

The new video eye image analysis algorithm, called the disk-fitting algorithm, was developed under the joint efforts of NASA and the University of Michigan. It tracks four dimensional ocular parameters, including the horizontal and vertical coordinates of pupil centers, pupil sizes, and torsion angles. The resolution of the measurements is less than 0.05 degrees for the pupil center tracking, and about 0.3 degrees for the torsion angles. Despite its capability to provide these extended measurements, the prototype VOMS is handicapped by its low computing power, geometric distortion, and poor user interface. Therefore, the prototype system has been operated in an off-line processing mode.

We propose to implement the new disk-fitting algorithm on a Power-PC based personal computer since a test proved that such PCs have adequate power for the real time execution of the disk-fitting algorithm. New image handling, geometric correction, and user-interface routines will be incorporated for a complete system.

Proper hardware components to constitute the proposed advanced video ocular system have been selected, and basic computing units were assembled. The base computing unit is a high-end desktop computer with four micro-processors. The framegrabber board to convert the analog video signal into digital data is implemented through the PCI interface bus to enhance image throughout. A preliminary version of the software to support multi-processing and hardware components such as the framegrabber and the frame memory has been built and tested. About 75 percent of the hardware-related task has been completed during this time.

The basic design of the new software interface has been accomplished. The new interface adopts the concept of a single instrument panel to ensure easy operation and maximum information delivery with minimum operation. Also, the basic research on the core algorithm decomposition for the multi-processing has been initiated. About 35 percent of all the software-related task has been completed.

The purpose of this project is to build a system that can accurately measure human eyeball movement. Since eye movement data carries important neurological information under certain circumstances through vestibulo ocular reflex, an apparatus providing accurate and noninvasive measurement, such as the proposed system, will become an invaluable tool for neuroscientists, no matter whether in space or on Earth.

Other than the originally proposed application area, the eye movement data can be used in many different capacities. Clinical medicine, hand-free computer/machine interface, and target aiming aid could benefit from automatic eye movement tracking. The system technology developed in this project will serve as a seed technology for expanding eye movement tracking into other application areas. The possibility of the spin-off of the proposed system to the commercial sector would be enormous.

FY96 Publications, Presentations, and Other Accomplishments:

Sung, K.J., Reschke, M.F., and Wood, S.J (abstract) A video ocular measurement system. American Institute of Aeronautics and Astronautics, Life Sciences and Space Medicine Conference, Houston, TX, AIAA Book of Abstracts, p. 119-120 (April 3-5, 1995).

Inflammatory and Mechanical Components of Muscle Injury

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-26-17-20
Initial Funding Date: 4/96
FY 1996 Funding: $176,570

Solicitation: 95-OLMSA-01
Expiration: 3/97
Students Funded Under Research: 14

Task Description:

The ability of personnel to function following space flight is limited by debilitating muscle weakness, pain, and inflammation that develop following return to gravitational loading. In this investigation, we will use the rat hindlimb suspension model to determine whether these injuries are attributable both to mechanical effects and to muscle damage by inflammatory cells. Our preliminary findings support the following, hypothetical injury mechanism. Muscle loading following periods of unloading causes mechanical damage to a small population of muscle fibers resulting in release of factors from injured cells that attract inflammatory cells. Neutrophils, which appear at higher concentrations in muscle at 2 hours following the onset of reloading, are the first inflammatory cells to arrive in the muscle. Neutrophils then induce further damage to reloaded muscle fibers by complement-mediated membrane damage and by reactive oxygen intermediates (ROIs). Most complement and ROI-induced damage occurs between 2 and 12 hours following initiation of reloading. At 12 hours reloading, phagocytic macrophages appear at elevated concentrations in muscle and invade injured fibers. At 24 hours reloading, macrophages associated with muscle repair appear at high concentrations.

We will test this hypothetical mechanism by manipulating loading conditions following hindlimb suspension so as to determine whether muscle damage during the peak injury period is attributable to mechanical loading or to inflammatory cell actions. We will also test the hypothesis by performing suspension/reloading on neutropenic rats to determine whether absence of neutrophils influences the severity of muscle damage. The role of specific, neutrophil-derived mediators of cell damage will also be assayed. Finally, we will use an *in vitro* model of muscle loading to test whether loading muscle in the presence of inflammatory cells results in more muscle damage than when muscle cells are loaded in their absence.

The results of this study can be of substantial importance to the mission of NASA by determining whether there is a central role for inflammatory cells in causing muscle injuries following space flight. Substantiation of this role for inflammatory cells will indicate that pharmaceutical approaches to control their activation during muscle reloading can ameliorate many of the debilitating effects of space flight on muscle.

We have tested mechanical and inflammatory components of muscle membrane damage by assaying the time course of membrane lesions and inflammatory cell invasion during modified loading. The data support the hypothesis that increased muscle loading stimulates neutrophil invasion into the muscle, and that neutrophil derived factors underlie most membrane damage that subsequently occurs. We are now testing this hypothesis

by generating neutropenic rats or rats in which we have blocked diapedesis of neutrophils into the muscle to determine whether this influences the extent of muscle fiber damage. We have produced a rabbit antibody to rat neutrophils that we have used for neutrophil depletions from suspended-reloaded rats and found that injections with this antibody results in muscles that are devoid of neutrophils following modified loading. We are currently analyzing the extent of fiber damage in the neutrophil-depleted muscles compared to controls. In our continuing investigations, we are evaluating the role of the complement system in muscle injury following modified loading by evaluating muscle injury in rats that have received intravenous administration of recombinant soluble complement receptor 1 (sCR1) that blocks activation of the alternative and classical complement cascades. We are also evaluating the contribution of reactive oxygen intermediates to muscle injury during modified loading in rats that have received intravenous administration of superoxide dismutase and catalase (SOD/CAT) to rats during hindlimb suspension and reloading to test whether superoxide contributes to muscle fiber injury during reloading. Because of the newly recognized importance in the relative concentrations of superoxide and nitric oxide (NO) in determining whether highly cytotoxic (e.g., peroxynitrite) or relatively benign ROIs are formed (e.g., Miles et al. 1996. *J. Biol. Chem.* 271: 40), we are now testing whether NO generated during muscle reloading contributes to inflammation and injury of muscle.

Our investigation, in which we are examining the contribution of inflammatory and mechanical factors to muscle injury following modified muscle use, is expected to be broadly relevant to understanding muscle injury and inflammation. If our findings substantiate our hypothesis that inflammatory cells are responsible for most muscle fiber injury that occurs during modified muscle loading, this will indicate that these muscle injuries can be controlled by the prophylactic use of anti-inflammatory drugs. This finding would be of practical value not only to returning astronauts, but also to individuals experiencing changes in musculoskeletal loading at 1-G. For example, this new knowledge would be of value in designing therapeutic approaches to control muscle injury during reambulation of patients following prolonged bedrest.

FY96 Publications, Presentations, and Other Accomplishments:

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Chang, W. J., Iannaccone, S.T., Lau, K.S., Masters, B.S.S., McCabe, T.J., McMillan, K., Padre, R.C., Spencer, M.J., Tidball, J.G., and Stull, J.T. Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy. Proc. Natl. Acad. Sci., 93, 9142-9147 (1996).

Cheung, E.V. and Tidball, J.G. (abstract) Ibuprofen inhibits muscle fiber invasion by macrophages during increased muscle loading. Med. Sci. Sports Exer., 28, S153 (1996).

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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 1996 Funding: $202,000
Students Funded Under Research: 2

Responsible NASA Center: ARC

Task Description:

Otolith signals of gravity and translational acceleration during head motion travel on vestibular afferents to central vestibular pathways to control vestibulo-ocular reflexes (VORs). VORs maintain eye position in space and stable vision during motion by generating compensatory eye motion. VORs are not isolated motor reflexes, but function with visual and somatic mechanisms that control head orientation on the body, and posture and locomotion. VORs are part of a sensorimotor orientation system permitting accurate, effective function in 3-D space. Normally, this system enables goal-directed motion, identification/following of visual targets, and identification/manipulation of external physical objects. Change in any part of the system (e.g., otolith function change in microgravity) impacts our ability to orient in 3-D. Understanding the nature of LVORs and how they adapt to environmental change motivates the proposed studies, and defines their relevance to NASA.

LVORs occur during motion along interaural (IA), naso-occipital (NO), and dorso-ventral (DV) head axes. LVORs include: 1) Two during motion along axes perpendicular to the line of sight, horizontal responses to IA-axis and vertical responses to DV-axis motion (both compensate for head motion); 2) Compensatory vertical and horizontal eye motion occurs during NO-axis motion (along axis parallel to the line of sight). Such LVORs are small or absent when gaze is straight ahead, and increase as gaze becomes more eccentric. Response phase reverses for gaze to right versus left, or up versus down; 3) For compensatory LVORs, target distance (vergence) is a potent influence; near targets require larger eye motion than more distant ones for similar head motion; 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, 2 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.

No additional studies were conducted during FY96. We have concentrated on completing data analysis and preparation of results of the adaptive plasticity experiments for publication. In addition, major effort was expended on preparation for Bion 11 experiments that were cancelled in March 1996 just prior to hardware shipping to Moscow for pre-flight experiments. Following that event, major effort was made to reinstall the laboratory at Ames that was to have been shipped to Moscow for the flight experiments.

Neural mechanisms underlying LVORS. To examine the role of irregular and regular afferents in modifying LVOR characteristics during linear and angular stimulation, electrical stimulation through labyrinthine stimulating electrodes was used 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. These experiments have been completed.

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 'de-conditioning' 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 (pp. 5-6 in "Sensorimotor Integration And Disintegration, A Workshop Sponsored By The National Aeronautics and Space Administration And The National Institute On Aging", 1992).

FY96 Publications, Presentations, and Other Accomplishments:

Minor, L., Tomko, D.L., and Paige, G.D. Torsional eye movements evoked by unilateral galvanic polarizations in the squirrel monkey. Proceedings of the 3rd Eye Movement Symposium, Tubingen, Germany, Harwood Academic Publishers (in press).

Schor, R.H. and Tomko, D.L. "The vestibular system" in "Vestibular-Sutonomic Regulation." Edited by: Yates, B. and Miller, A. CRC Press/Boca Raton, FL, Chapter 1, (1996).

Tomko, D.L. and Clifford, J.O. (abstract) Effect of spaceflight on vestibulo-occular reflexes (VORs) during angular head motion. Neurosci. Abstr. (1996).

Pharmacological Intervention to Prevent Disuse Osteopenia

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Funding:

Project Identification: 199-18-17-22
Initial Funding Date: 1/96
FY 1996 Funding: $110,188

Solicitation: 95-OLMSA-01
Expiration: 12/96
Students Funded Under Research: 2

Task Description:

We propose that disuse osteopenia results in large measure from disturbed expression of skeletal signaling molecules (e.g., TGF-ß). These signaling molecules are not exclusively regulated by weight bearing; they serve as intermediates in the signal transduction pathways for many physiological regulators of bone metabolism (e.g., calcium regulating hormones). As a result, it should be possible to bypass the mechanoreceptor step in the signal transduction pathway induced by dynamic weight bearing. This goal could be accomplished with pharmacological agents which modulate expression of the same skeletal signaling molecules (e.g., growth factors) as physical activity. Specifically, we propose that pulsatile administration of parathyroid hormone (PTH), will prevent disuse osteopenia. Furthermore, we predict that dynamic weight bearing and PTH each act to stimulate bone formation by regulating the expression of transforming growth factor-ß (TGF-ß) in skeletal tissues.

The goals of the two year proposal are to: 1) establish a method to administer PTH during spaceflight which does not require astronaut intervention, and 2) determine if the anabolic action of PTH on bone formation requires weight bearing. The first goal will be accomplished by implantation of Alzet osmotic pumps that are loaded to deliver pulsatile release of PTH. The second goal will be realized by testing the effects of PTH on bone formation in hindlimb unloaded rats. In the latter study, the effects of the hormone on unloaded hindlimbs will be compared to the effects on the loaded forelimbs.

The first specific aim of the proposed research, which was to establish a method to administer pulsatile parathyroid hormone (PTH) during space flight that does not require intervention by the crew, was accomplished. Timecourse studies revealed that discontinuous administration of PTH can be as effective as subcutaneous daily injection of the hormone in increasing osteoblast number and bone formation. The second specific aim of the proposal, which is to determine the efficacy of PTH in preventing cancellous osteopenia in an unloaded limb, can now be tested.

Although these studies were directed at developing a method to administer PTH to laboratory animals during space flight, the results are relevant to human disease. PTH treatment holds great promise as a therapy to treat several common forms of bone loss including postmenopausal osteoporosis. Potential problems of PTH therapy include cost, detrimental side effects, and the requirement to endure frequent injections. The important side effects of PTH treatment consist of hypercalcemia and skeletal abnormalities.

It is known that continuous exposure to PTH is detrimental, but the precise therapeutic "window" of exposure is not known. Our results are important because they demonstrate that PTH cannot remain in circulation for intervals much in excess of 1 hour without serious consequences. Furthermore, we have shown that it is possible in theory to administer PTH discontinuously using a simple implantable device. As a consequence, it may be possible to evoke the full therapeutic actions of PTH without the need for frequent injections.

FY96 Publications, Presentations, and Other Accomplishments:

Dobnig, H. and Turner, R.T. (poster Pl-916) Programmed intermittent sc infusion of parathyroid hormone in sexually mature rats: Effects on bone and mineral metabolism. Scientific Program of the 10th International Congress of Endocrinology, San Francisco, CA (June 12-15, 1996).

Reconstructions and Representations of Cerebral Cortex [Human Brain Project]

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Funding:

Project Identification: n/a
Solicitation:
Initial Funding Date: 9/94
Expiration: 4/99
FY 1996 Funding: $20,000
Students Funded Under Research: 6
Joint Agency Participation: NIH and Human Brain Project

Task Description:

Neuroscientists have obtained vast amounts of experimental data about the organization and function of the cerebral cortex in non-human primates, especially the macaque monkey. To cope with this flood of information, new tools and strategies are necessary in order to adequately analyze and communicate these findings. To this end, we propose a collaborative effort that brings together scientists with complementary expertise in neuroanatomy and image processing and capitalizes on access to high-performance parallel computing resources and high-speed networking capacities. Our common goal is to develop and apply a family of interrelated computer graphics programs to be used for representing information about cortical structure and organization. The integrated system will allow visualization of three-dimensional (3-D) reconstructions of the entire cerebral hemisphere that are based either on volumetric representations or on selected surface contours. These reconstructions will be used to display information about the location of different cortical areas as well as data from specific experimental procedures. To compensate for the marked differences between individual brains, we will develop warping algorithms that can accurately transform one brain into the shape of another. These transformations will be based on probabilistic approaches to shape modeling that have had considerable success in other domains of biology. We will also develop computerized techniques for making unfolded representations of the cortex. These techniques will be used to generate comprehensive, easily updatable summaries of different schemes for the layout of various areas throughout the cerebral cortex. These will in turn be used as the framework for a graphically oriented database of the connectivity of different areas. Collectively, these approaches will greatly enhance the accuracy, speed, and flexibility with which many types of information about cortical organization can be represented and communicated. In addition, it will provide a much needed framework for more accurate comparisons with the human brain.

Our collaborative effort to develop and apply new approaches to the mapping of primate cerebral cortex has made significant advances on several fronts. Much of our effort focuses on the fact that the cerebral cortex is a highly convoluted surface which needs to be manipulated in various ways (e.g., warped or flattened) in order to study its

functional organization in individual brains and to compare its structure and organization across different individuals. Using a flattening algorithm developed the preceding year, we have generated a surface-based atlas of the human cerebral cortex that is based on the brain of the Visible Man (Visible Human Project, NLM). This atlas is particularly useful for evaluating the large amounts of data emerging from functional brain imaging studies. For example, we have already gleaned new insights concerning the functional organization of human visual cortex by a combined analysis of published PET imaging studies. On another front, we have continued our refinement of shape-based deformation algorithms that are constrained by the topology of the cortical surface as they deform one brain to match the shape of another. One important variant of this strategy involves shape-based deformations applied to cortical flat maps, which is computationally less expensive than volume deformations. We have demonstrated using flat maps of both monkeys and humans how this can be used to transform one hemisphere to conform to the shape characteristics of another hemisphere using geographical and/or functionally based landmarks. This strategy is particularly promising when attempting to retain the high spatial resolution available with functional MRI (fMRI), which is lost or corrupted when data are transformed to an atlas using conventional 3-D warping algorithms. Finally, we have developed new algorithms capable of automatically analyzing the geometry of the cortical surface, including one algorithm that can trace gyral and sulcal landmarks on the cortical surface and another algorithm that can determine geodesic (shortest-distance) trajectories. These will be important additions to our suite of tools that allow flexible, high fidelity manipulations of cortical surfaces. Finally, we have continued the development of 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.

FY96 Publications, Presentations, and Other Accomplishments:

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Investigation Of Laser-Polarized Xenon Magnetic Resonance

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Funding:

Project Identification: 199-04-17-16
Initial Funding Date: 05/01/96
FY 1996 Funding: $116,391

Solicitation: 95-OLMSA-01
Expiration: 4/30/99
Students Funded Under Research: 4

Task Description:

We propose ground-based investigations of a new biomedical diagnostic technique: the inhalation and magnetic resonance (MR) of laser-polarized ^{129}Xe (xenon) gas. Laser-polarized ^{129}Xe MR may allow the imaging and spectroscopy of human body structures and functions that have heretofore been poorly resolved by conventional proton MR, either because of low signal level and structural artifacts (e.g. the lung and lipid membranes), low signal contrast (e.g. differential perfusion studies), or poor tissue specificity (e.g. chemical shift MR). Laser-polarized ^{129}Xe MR is a recently demonstrated technology that may have important biomedical applications, including: (i) detailed lung imaging; (ii) the measurement of blood flow to tissue (perfusion); (iii) the characterization of lipid membrane integrity (e.g. in multiple sclerosis); (iv) chemical shift imaging and spectroscopy; and (v) the study of anesthesia. In addition, ^{129}Xe laser-polarization occurs external to the body, prior to the inhalation of the gas. As a result, at very low magnetic fields (~0.001 tesla) the signal-to-noise ratio (SNR) of laser-polarized ^{129}Xe MR can be much larger than one, whereas the SNR of proton MR is less than one. The creation of such very low magnetic fields is practical in a small, low-power device. Therefore, since the production of laser-polarized gas should be possible in a small, low-cost package, very-low-field ^{129}Xe MR may enable both portable ground-based and practical space-based biomedical MR systems.

The present NASA grant (NAGW-5025) supports the development of a new biomedical diagnostic technology — the magnetic resonance imaging (MRI) and spectroscopy (MRS) of laser-polarized noble gases. The research aims of the present grant fall into three general areas: (i) development of noble gas polarization systems; (ii) biomedical investigations using polarized noble gas in conventional (high magnetic field) MRI/MRS systems; and (iii) development and application of a low magnetic field system for polarized noble gas MRI/MRS.

Below is a description of what has been accomplished in FY96 during the partial first year of the present grant (5/1/96 to 9/30/96).

• Investigation of ventilated animals.

We observed ^{129}Xe MR spectra in the thorax of living rats breathing the laser-polarized gas, as well as images of polarized ^{129}Xe gas inside the rat lung. These were the first reported in vivo ^{129}Xe spectra. We observed three

well-resolved ^{129}Xe tissue resonances, in addition to the gas resonance in the lung gas space. Once xenon inhalation was stopped, the three ^{129}Xe tissue resonances were observed to decay with different time constants ranging from 11 to 50 seconds, i.e., longer than the blood circulation time in the animal.

• Investigation of ventilated humans.

We reported the first polarized noble gas image obtained in a human: a simple image of the inside of the oral cavity of a person inhaling polarized ^{129}Xe. This image served as a first-step demonstration of the new technique of laser-polarized noble gas MRI for humans.

Subsequently, we constructed and tested signal acquisition coils appropriate for laser-polarized ^{129}Xe MR of ventilated humans. Surface and birdcage coils for the head and thorax were developed; these coils are double-tuned for both proton and ^{129}Xe Larmor frequencies at 1.5 tesla.

• Model calculations.

We reported the first model calculation of the build-up of inhaled, polarized ^{129}Xe in human tissues. This model assumes the steady inhalation of polarized ^{129}Xe gas mixed with air, and accounts for ^{129}Xe depolarization in the lung, blood, and tissue of interest. With the caveat that important parameters such as the ^{129}Xe T_1 times in blood and tissues are not well known *in vivo*, we calculated that at 1.5 tesla the ^{129}Xe MR signal-to-noise ratio in human brain tissues is ~ 1-10% of that from proton MR in typical water-rich tissue. At low field (~ 0.01 tesla) the relative magnitudes of the ^{129}Xe and water proton MR signal-to-noise ratios are expected to reverse.

• Development of a low-field solenoid.

We constructed a solenoid to provide a stable, homogeneous magnetic field < 100 gauss, with first through fourth order static-gradient correction coils. Pulsed gradient coils for 2D spectroscopy and imaging are currently being developed.

This research supports the development of new biomedical diagnostic technology — MRI and MRS of laser-polarized noble gases. Large nuclear spin polarizations (> 10%) can be created in dense samples of the spin 1/2 noble gases (^3He and ^{129}Xe) using the technique of spin-exchange optical pumping. Such large polarizations greatly enhance the magnetic resonance detection sensitivity of ^3He and ^{129}Xe, enabling high resolution gas space imaging, studies of gas diffusion in porous and granular media, and investigations of fluids using the soluble ^{129}Xe species. Polarized ^3He and ^{129}Xe can be benignly inhaled by humans with minimal loss of spin-polarization (T_1 ~ 5 to 50 seconds, depending on the organ and tissue) and then detected with MRI/MRS. Potential biomedical applications include improved lung imaging (important for emphysema diagnosis); the imaging of lipid membranes in the brain (useful in the diagnosis of multiple sclerosis and in research on brain function); and better measurement of blood flow to tissue (important for stroke and ischemia diagnosis, and also useful in research on brain function). In addition, noble gas laser-polarization occurs external to the body and does not require a large magnetic field. As a result, at low magnetic fields (~ 0.01 tesla) the signal-to-noise ratio (SNR) of laser-polarized noble gas MRI/MRS can be much larger than the SNR of proton MRI/MRS. Low-field noble gas MRI/MRS may thus be practical in a small, low-power device and enable both portable ground-based and practical space-based biomedical MRI/MRS systems. Our biomedical investigations using polarized noble gas MRI/MRS are in collaboration with the Magnetic Resonance Division of the Brigham and Women's Hospital, headed by Dr. Ferenc Jolesz.

FY96 Publications, Presentations, and Other Accomplishments:

Albert, M.S., Tseng, C.H., Williamson, D., Oteiza, E.R., Walsworth, R.L., Kraft, B., Kacher, D., Holman, B.L., and Jolesz, F.A. Hyperpolarized ^{129}Xe MR imaging of the oral cavity. J. Magn. Reson., Series B 111, 204 (1996).

Peled, S., Jolesz, F.A., Tseng, C.H., Nascimben, L., Albert, M.S., and Walsworth, R.L. Determinants of tissue delivery for ^{129}Xe magnetic resonance in humans. Magn. Reson. Med., 36, 340 (1996).

Sakai, K., Bilek, A.M., Oteiza, E., Walsworth, R.L., Balamore, D., Jolesz, F.A., and Albert, M.S. Temporal dynamics of hyperpolarized ^{129}Xe resonance in living rats. J. Magn. Reson., Series B 111, 204 (1996).

Tseng, C.H., Oteiza, E.R., Walsworth, R.L., Albert, M.S., Nascimben, L., Peled, S., Sakai, K., and Jolesz, F.A. Biological studies with laser-polarized ^{129}Xe. DAMOP Meeting of the American Physical Society, Ann Arbor, MI (May 15-18, 1996).

Walsworth, R.L., Stoner, R.E., Tseng, C.H., Wong, G.A., and Oteiza, E.R. Large-scale production of laser-polarized ^{129}Xe: Comparison of laser-diode-array and Tisapphrie optical pumping. DAMOP Meeting of the American Physical Society, Ann Arbor, MI (May 15-18, 1996).

Adapting to Altered Gravity and Vision

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Funding:

Project Identification: 199-16-12-34
Solicitation: 93-OLMSA-07

Initial Funding Date: 10/95
Expiration: 9/98

FY 1996 Funding: $137,000
Students Funded Under Research: 6

Responsible NASA Center: ARC

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 (+two 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 seven days of continuous +two Gz, by means of centrifugation, and seven 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.

I. Studies of Hypergravity

In the last year, members of the research team completed several studies examining the effects of an adaptation to centrifugally-produced hypergravity.

It was found (a) that hypergravity causes a visual target to appear progressively higher (the "elevator illusion") as Gz increases from 1.0 to 1.5 to 2.0, (b) that the illusion is greater when the target is presented in an otherwise

dark setting, (c) that a pitchbox viewed during hypergravity strongly biases the visual target settings in the same direction as the box pitch and (d) that gaze direction is affected by increasing Gz in the same manner as the elevator illusion. These results indicate that changes in visually perceived elevation, whether from the elevator illusion or a pitched visual surround, are based on misinterpreted gaze direction. This research was reported by Cohen (1996).

In another investigation, rats were exposed to 7 d of chronic hypergravity (2.0 Gz), alternative with 7 d of 1-G, for a total of four alternations (i.e., 8 wk). Immediately after a given period of centrifugation, subjects were tested on their ability to readapt to normal gravity in terms of the righting reflex (after an unexpected drop from a supine position into a vat of water) and subsequent swimming behavior. Dual adaptation was seen in terms of faster recovery of the righting reflex after the last hypergravity exposure period than after the first. This research was reported by Corcoran, Daunton, Fox, Welch, and Wu (1996)

II. Studies of Adaptation of the Vestibulo-ocular Reflex (VOR) and Apparent Concomitant Motion (ACM)

In the last year, members of the present research team completed several studies of VOR and ACM adaptation, the results of which lay some of the groundwork for the studies proposed in the next section. One series demonstrated that (a) VOR adaptation can be produced by both active and passive bodily rotation, (b) dual adaptation occurs only for active rotation, and (c) adaptive generalization did not occur for either active or passive rotation. In a second series, evidence was found for (a) dual adaptation of ACM in which the discriminative stimulus was the frequency of head turing (.5 vs. 2.0 Hz) and (b) the operation of two mechanisms in the elimination of ACM adaptation. The results of some of these studies were reported by Bridgeman, Welch, and Williams (1996), Bridgeman, Williams, and Welch (1997), Post (1997), Post and Welch (1996), Post and Welch (1997), Welch, Bridgeman, Williams, and Semmler (1997), and Williams, Bridgeman, Welch, and Strain (1997).

During the last year, members of the current research team completed several studies of the spatial effects of and adaptation to pitched visual arrays. This research obtained evidence (a) for the occurence of errors in pointing at targets located in pitched visual environments and their elimination by adaptation, (b) that the effect of pitch on VPEL is determined by retinal, rather than perceived, image size of the displays, (c) that the effects of pitched arrays on VPEL cannot be predicted on the basis of the perceived pitch of the array, (d) that visual displays made up of random dots influence VPEL just as much as those composed of vertical edges, (e) walking back and forth in a large pitched environment (a "pitchroom") while fixating on the back wall reduces its effect on VPEL, (f) the effect of a pitched visual surround on VPEL is accompained by an "optostatic response" in which the eyes unconsciously turn in the same direction as the pitch by an amount that is almost perfectly correlated with the VPEL shift, and (g) VPEL in a pitched surround is best described as a linear summation of the weighted independent contributions from a body-referenced mechanism and a visual mechanism given by the orientation of the background array relative to the head. The results of some of these studies were reported by Post and Welch (1996), Post, Welch, and Clark (1996), Stoper, Randle, and Cohen (1996), and Welch and Post (1996).

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.

FY96 Publications, Presentations, and Other Accomplishments:

Bridgeman, B., Welch, R.B., and Williams, J. Dual adaptation, but no adaptive generalization of the human VOR. Poster presented at Association for Research on Vision and Opthalmology Day, University of California, Berkeley, CA. June 1996.

Cohen, M.M. Elevator illusion and gaze direction in hypergravity. Av., Space & Environ. Med., 67, 676 (1996).

Corcoran, M., Daunton, N., Fox, R., Welch, R.B., and Wu, L. Effects of repeated chronic exposures to 2G on disruption of air righting reflex in the rat. Poster presented at American Society of Gravitational and Space Biology annual meeting, Charlotte, NC. October 1996.

Post, R.B. and Welch, R.B. The role of retinal versus perceived size in the effects of pitched displays on visually perceived eye level. Perception, 25, 853-859 (1996).

Post, R.B. and Welch, R.B. Simultaneous opposite adaptations of apparent concomitant motion contingent on frequency of head motion. Paper presented at the Annual meeting of the Association for Research on Vision and Opthalmology, Fort Lauderdale, FL. 1996.

Post, R.B., Welch, R.B., and Clark, V.D. Visually perceived eye level: Effects of pitch perception and eye position. Paper presented at the meeting of the Psychonomic Society, Chicago, IL. November 1996.

Stoper, A.E., Randle, J., and Cohen, M.M. The effect of environmental pitch on perceived optic slant and eye level: Lines vs. dots. Paper presented at the 19th European Conference on Visual Perception, Strasbourg, France. September 1996.

Welch, R.B. and Post, R.B. Accuracy and adaptation of pointing in pitched visual environments. Perception and Psychophysics, 58, 383-389 (1996).

Altered Gravity Locomotion Using Differential Pressure

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Project Identification: 199-26-12-40
Initial Funding Date: 2/95
FY 1996 Funding: $38,000

Solicitation: 93-OLMSA-07
Expiration: 2/97
Students Funded Under Research: 1

Responsible NASA Center: ARC

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 (UBPP) 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 0.25-G to 1.5-G. 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 hypogravity/hypergravity locomotion simulator is awaiting rating by the Human Occupancy Review Board. In the meantime we have determined that the insole force sensors that we had proposed using to obtain vertical ground reaction forces will not work in the changing pressure environment of the chamber. We are now

modifying an instrumented treadmill used for underwater studies at the Ames Research Center for use inside our chamber.

We believe the primary cause of bone loss in space is the reduction in the 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 system are their inability to support patients comfortably when supporting a significant portion of body weight and their 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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Project Identification: 199-26-12-35
Initial Funding Date: 2/95
FY 1996 Funding: $94,000

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 0

Responsible NASA Center: ARC

Task Description:

Exercise has not been entirely successful in maintaining bone mass in cosmonauts during space flight. One reason may be the lack of quantitative data on normal daily activity and exercise from which to develop a basis for selecting and optimizing specific exercises. Experimental studies have identified peak cyclic forces, number of loading cycles, and loading rate as contributors to the regulation of bone density and structure. We have hypothesized that bone density and structure are maintained by daily tissue effective stress histories generated by physical activity. Furthermore, application of these ideas to the calcaneus and lower limbs suggests that bone density and long bone structural integrity in these regions may be quantified in terms of the daily histories of the ground reaction force (GRF). The objectives of this proposal are: (1) to examine the relationship between the spatial distribution of mineral at a long bone cross-section and the cross-sectional flexural properties, i.e., rigidities; (2) to quantify physical activity in terms of the daily history of ground reaction forces; and (3) to examine whether calcaneal bone density and long bone (tibia) cross-sectional areal properties are correlated to the daily history of ground reaction forces. Additionally, we have begun a collaborative effort with the Department of Veterans Affairs (Dr. Gary Beaupré) and Department of Radiology, Stanford University (Dr. Sandy Napel) to develop state-of-the-art surface-based registration algorithms for registration of serial calcaneal scans (partial VA support). (Because of a 1/3 funding cut in proposal budget, a large collaborative third-year human subjects study with Dr. Robert Marcus was postponed.) *Study #1:* Male and female tibiae will be scanned and strain-gaged by our methods. Geometric properties obtained from the scans will be correlated to flexural rigidities obtained from strain gage data. *Study #2:* Daily activity levels of 25 non-exercising subjects will be measured for three days using the GRF system, log books, and pedometers. Calcaneal bone density and long bone properties will be correlated to the mean daily history of the ground reaction force using parameters derived from our model of bone adaptation, pedometers, as well as other functions of daily GRF history obtained from the loggers. Expected results. *Study #1:* Since densitometry measures the distribution of the load-carrying mineral, we expect our results to be highly correlated to measurements computed from *in vitro* surface strain measurements. *Study #2:* We expect the correlation of bone density and structure to activity level to improve with increasingly quantitative measures of physical activity with a best fit using our model and GRF peak cycles and loads from the logger.

As part of this proposal, we have developed and validated a technique for obtaining the principal area moments of inertia and the principal axes along the length of long bones. We have applied the method to embalmed tibiae

in vitro and stripped of soft tissue. In addition we have strain-gaged multiple cross-sections of these bones, loaded them in cantilever bending and recorded the surface strains and applied load. By applying a method developed by Gies and Carter, we obtained the principal flexural rigidities and principal angles for each section. These were then compared to the principal area moments of inertia and angle from non-invasive densitometry. The angles were the same to within a few degrees and principal flexural rigidities correlated highly with principal area moments (r2 = ~0.96), indicating to us that flexural properties are determined (at least in these embalmed bones) by the spatial distribution of bone tissue independent of the local apparent density. As an outgrowth of this work we applied the technique to small (rat) bones using the Hologic QDR's small animal collimator as the x-ray energy source. The initial phase of this work was reported for the end of year one. In the second year we improved our small animal algorithms, noise reduction and image processing approach. While the pixel spacing of the small animal software is only 0.125 mm, the beam width is on the order of the size of rat bone diameters causing a "smearing" of the density profile of a long bone cross-section. In year two we re-derived our beam deconvolution filter based on the beam profile of the QDR 1000W. These methods differed from our earlier method using a point spread or impulse function. Processing by deconvolution filter improved the accuracy of determining principal moments in aluminum phantoms the size of rat femurs from 10-20% error to 2.0-2.5% error. This software is now available for general use provided scanner beam characteristics of similar machines in different laboratories are the same. Otherwise, a different filter would need to be derived from beam data. In year two we also redesigned and reconfigured our portable ground reaction force-monitoring system to be usable by the general public as compared to our earlier prototype. Prompting of the user and communication of commands is now done through a set of LEDs and unique tonal sequences generated by an internal speaker. The unit is smaller and housed in a fanny pack. Ten complete units are now functioning with another fifteen in production. We recently completed a three-day monitoring study in 24 men and women with units. Laboratory force plate calibration of the sensor and hourly in-the-field calibrations at the prompting of the unit indicate that our accuracy is on the order of five-eight% over the full range of expected daily loading (zero to three body weight). This relatively high accuracy, for an insole sensor, is achieved by post-processing insole data with a special non-linear (one parameter) model of sensor response. Furthermore, the parameter varies with wear of the sensor necessitating our tracking the wear as well. Results from the monitoring study are being processed currently and will be reported. Finally, in collaboration with the Department of Veterans Affairs Palo Alto and Stanford University Radiology, we have begun development of a program to apply quantitative computed tomography to the calcaneus for mapping changes in volumetric bone density with time. State-of-the-art registration algorithms have been developed and reported. We are now working on corrections for beam hardening to improve the accuracy of the method.

The primary goal of our research is to clarify the relationship between the musculoskeletal tissue stress (strain) histories developed during normal daily activity and functional adaptation of musculoskeletal tissue. This proposal addresses the both research and technical goals that NASA, the National Research Council, the National Institute of Health, and the National Institute on Aging (NIA) have identified as critical to space biology and medical science and health care on Earth (in *A Strategy for Space Biology and Medical Science for the 1980s and 1990s*, National Academy Press, 1987; *The Effects of Space Travel on the Musculoskeletal System*, NIH Publ. No. 93-3484). In addition the NIA has targeted "Frailty" (age-related biomechanical factors affecting physical performance), "Osteoporosis" (non-estrogenic factors affecting bone loss and bone strength), and "Physical Exercise" (effects of exercise on bone and muscle mass and function) as high priority research areas. Specifically, we have hypothesized that bone loss in space and bone loss with age on Earth are in large part due to reduced daily cumulative loading in space and declining activity level with age on Earth. The objective of this research is to integrate our mathematical model of bone adaptation. Novel instrumentation to monitor daily lower limb musculoskeletal loading and advanced bone imaging methods using projected radiography (DXA) and quantitative computed tomography (QCT) will be used to study bone adaptation non-invasively in humans. We are focusing on the calcaneus and lower limb bones as model sites loaded by muscles and joint forces that are predominantly determined by the external ground reaction force (GRF). These bone sites are also most significantly affected by long duration space flight. We have selected the calcaneus as a primary model bone site because (1) it is loaded by daily ground reaction forces which we now can measure; (2) it is a peripheral bone site and therefore more easily and accurately imaged; (3) it is composed primarily of cancellous bone; (4) it is a highly relevant site for monitoring bone loss in astronauts; and (5) it is clinically relevant as the bone of choice

for diagnostic ultrasound measurement. We believe our ground-reaction-force-monitoring instrumentation, bone imaging techniques to examine structural changes in lower limb long bones from planar densitometry and QCT in the calcaneus, developed in collaboration with colleagues at the Palo Alto Veterans Administration and the Department of Radiology at Stanford, represent state-of-the-art contributions to the field. Areas of future applicability include the study of bone adaptation to chronic (e.g., spinal cord injury) and acute (e.g., space flight) disuse, exercise, drug therapy and aging.

FY96 Publications, Presentations, and Other Accomplishments:

Anderson, F.C., Ziegler, J.M., Pandy, M.G. and Whalen, R.T. "Solving large-scale optimal control problems for human movement using supercomputers" in "Building a Man in the Machine: Computational Medicine, Public Health and Biotechnology, Part II." Edited by: Witten, M. and Vincent, D.J. World Scientific, pp 1088-1118, (1996).

Breit, G.A. and Whalen, R.T. System for monitoring ground reaction forces for quantification of daily activity. Tech. 2006 Conference, October, Anaheim, CA (1996).

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Morey-Holton, E.R., Whalen, R.T., Arnaud, S.B., and van der Meulen, M.C. "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: Blatteis, C.M. and Fregly, M.J. Oxford University Press/New York City, NY, pp 691-719, (1996).

Napel, S., Yan, C.H., and Whalen, R.T. Serial scanning and registration of high resolution quantitative computed tomography volume scans for the determination of local bone density changes. NASA Final Report: NCC2-5088, (1996).

Yan, C.H., Beaupré, G.S., and Whalen, R.T. (abstract) Precise and accurate standard for multimodality and serial registration method evaluations. Proceedings of the 82nd Annual Meeting of the Radiological Society of North America Radiology, 201 (P293) (1996).

Renal-Endocrine Response to Gravity and Sleep Disruption

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Project Identification: 199-18-17-14
Solicitation:
Initial Funding Date: 9/94
Expiration: 8/96
FY 1996 Funding: $119,727
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, and 2500 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 six degree head-down tilt for five days, while simultaneously undergoing forced desynchrony, and then the above protocol will be repeated.

During the past year we have overcome a number of substantial problems in the complete implementation of this project. The first difficulty was the duration of the proposal as original designed. The original design had six individuals hospitalized for 30 days while they underwent the simulated microgravity, and then the disrupted sleep portions of the protocol. This long duration resulted in incomplete studies and poor compliance. It was, therefore, necessary to develop a different format. On careful analyses of our protocol's aims, it became clear that the study could be performed in two parts. Each part could be performed in the same or different individuals. One part would look at microgravity, and the second would examine sleep disruption. Thus, each phase would consist of approximately a 13-15 day admission to the Intensive Physiologic Monitoring unit, rather than the previous 30 day admission. Therefore, instead of six studies to be completed during the course of the study, it will be necessary to complete 12 studies, each half in length.

Because we assumed the microgravity phase of the protocol would be easier to accomplish, we have focused our efforts on this part of the project. Again, there have been substantial difficulties in implementing the project. First, to enroll women in the project, we need to enroll them at a fixed time in their menstrual cycle. This has resulted in a non-completion rate of nearly 70% for women. The second problem was the prolonged period of supine posture, to which many of our subjects objected. Thus, our screening procedures have, of necessity, needed to be increased because of the relatively low yield of appropriate study volunteers. In the current year, two subjects have completed the protocol and no specific side effects or difficulties have developed, except for profound symptoms of postural hypotension in the immediate post-supine posture phase of the study. This has required a more gradual assumption of the upright posture part of the protocol, to minimize the number of individuals who had a syncopal episode.

Because of the difficulty we have encountered in enrolling normal subjects in a sequential fashion, we have now activated both parts of the protocol simultaneously. Therefore, our initial approach is to recruit subjects into the supine phase of the protocol. If the subject objects to this phase, we will then shift them to the disrupted diurnal variation study. We believe in this way we will maintain our original target of having two patients admitted each month, with anticipated completion of the protocol by May 1997.

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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Funding:

Project Identification: 199-26-17-06
Initial Funding Date: 5/94
FY 1996 Funding: $134,000

Solicitation:
Expiration: 5/97
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 is associated with an alteration of collagen cross-linking. Our underlying hypothesis is that the perturbation of the mineralization process in reaction to weightlessness and upon return is caused by changes of collagen fibrillar structure arranged and stabilized by intermolecular cross-linking. Our continuous research on the characterization of collagen cross-linking and fibrillar structure in the various connective tissues (mineralized as well as nonmineralized, normal as well as pathological) have been and will be the basis for this study. As a simulation of weightlessness, a mature rat model of the one-legged long-term immobilization will be employed. Two groups of adult rats will be prepared for this study: a control (sham) and a group which will be subjected to immobilization (15 weeks) and subsequent reambulation (20 weeks). During the course of this study, bones (femurs) of the following conditions will be studied: 1) normal (control); 2) unloaded and subsequent reambulation; and 3) overloaded and subsequent reambulation. Due to the differences in the turnover rate, the cancellous bone of femur metaphyses and compact bone of diaphyses will be collected separately and subjected to a detailed characterization. The molecular packing structure of type I collagen fibril will be investigated by quantifying the intermolecular cross-links and their precursor aldehydes at their specific molecular loci within the fibril. The analyses of bone mineral will include the content of mineral and its crystallinity measured by electron paramagnetic resonance (EPR) spectroscopy. The mechanical properties of bone will be evaluated to seek any correlation of these properties to the nature of bone mineral and collagen fibrillar structure. These data would provide insight into a regulatory role of collagen structure in the deposit and growth of mineral crystals during bone structural adaptation to various mechanical stresses.

In order to study the spatial relationship between collagen fibril and its mineralization, we have been continuing our study on differential collagen post-translational modifications using mineralizing turkey tendons as a model. In the previous FY95 Task Progress, some preliminary data was given. We have further analyzed collagen cross-links in the three compartments: peripheral-(Peri-, never-mineralized), inner mineralized- (Mineral.) and inner nonmineralized (Nonmin.) as a function of age using 13 to 91 week old turkeys. Throughout the animal's age, the major cross-link of Peri was a tetrafunctional reducible cross-link, dehydro-histidinohydroxymerodesmosine (HHMD) (0.5-0.8 mole/mole of collagen). A nonreducible cross-link, pyridinoline (Pyr) was present in a low concentration (0.1-0.2m/m col). In the case of Nonmineral., however,

the major cross-link was Pyr (0.6-0.8 m/m col) throughout the animal's life and the reducible cross-links dehydro-dihydroxylysino-norleucine (DHLNL) and dehydro-hydroxylysinonorleucine (HLNL) diminished from 0.3 to 0.05 m/m col during 13 to 30 weeks old of age and remained low thereafter. HHMD was 0.2-0.3 m/m col level throughout the animal's life. In the case of Mineral., significantly different chemistries were found. Pyr was very high at 13 weeks of age (0.8 m/m col) but diminished quickly to a 0.3-0.4 level when the animals reached 30 weeks old and remained the same level thereafter. A dramatic difference was seen in deoxypyridinoline (D-Pyr). This particular cross-link was present essentially *only in the Mineral. portion* and its concentration increased from 0.03 to 0.3-0.4 m/m col when mineralization proceeded (13 to 30 weeks of age). A significant amount of free Hylald was found only in this fraction. It could be the result of dissociation of DHLNL by physical force brought about by mineralization (Yamauchi et al 1992). In addition, HHMD, which was the major cross-link in Peri (never mineralized), was found to be minute (0.02 to 0.1) in this compartment. The fact that the major cross-link in Peri. collagen is HHMD supports our hypothesis concerning the down-regulatory role of this cross-link in mineralization. These data clearly indicate that new type I collagen is deposited prior to/during mineralization replacing the preexisting one, at least in part, and the post-translational chemistries (hydroxylation of lysine and cross-linking) of this new type I collagen are distinct from those of the nonmineralized portion. Furthermore, for a comparative purpose, we obtained other tissues from turkeys (neck tendon-never mineralized, articular cartilage and cortical bones) and performed cross-link analysis. The results showed that the cross-linking pattern of neck tendon collagens was similar to that of Peri.-collagen (abundant in Lys^{ald}-derived cross-links, such as deH-HLNL and deH-HHMD and no deoxypyridinoline). The significant difference between these two never-mineralizing tendon collagen is the content of pyridinoline cross-link (Peri contains significant amount of pyridinoline, >0.4 mol/mol collagen, but neck tendon does not, <0.04 mol/mol of collagen). The cross-linking chemistry of cartilage was similar to that of Nonmineral.-collagen (high pyridinoline but no deoxypyridinoline). The cross-linking pattern of cortical bone collagen was similar to that Mineral.-collagen (high deoxypyridinoline). These data indicate that there are at least three cell phenotypes making post-translationally distinct type I collagen (bone-, cartilage-, and tendon-like collagen). The potential regulatory roles of cross-linking in mineralization will be further pursued by determining their molecular distribution in the fibril.

We have made progress in our studies on bone collagen cross-links in the toothless (tl/tl) osteopetrotic rats and the effects of the 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 was 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. The content of mineral and its crystallinity of bones obtained from both groups were measured. The latter was analyzed by electron paramagnetic resonance spectroscopy (ESR) in collaboration with Dr. Dziedzic-Goclawska (consultant). There were no significant differences in bone mineral content between two groups, bu*t a significant decrease in the mineral crystallinit*y was observed in all bones analyzed of tl/tl mutants when compared to the control. During the course of this study, we accidentally found that the cross-linking chemistry in long bone collagen was clearly different from that of parietal bone. In all experimental groups examined, the concentrations of pyridinoline were higher than deoxypyridinoline in long bones (both cancellous and compact) while the ratio was reversed in the parietal bones indicating the difference in the degree of lysine hydroxylation. The same observation was made in another osteopetrotic rat mutation (op/op, Fatty-Orl-op strain). Whether this difference is due to the difference in the mineralization process (endochondral vs. intramembranous ossification) and/or mechanical stress (load-bearing vs. nonload-bearing) is yet to be studied. Currently, we have been analyzing those bones from different species (porcine and bovine) to determine if this difference generally exists. The difference (lysine hydroxylation) could have significant biological implications, because it could lead to a different cross-linking pathway and also could alter the fibril growth due to the different degree of glycosylation (hydroxylysine is the only known glycosylation site of a collagen molecule). These chemical and structural differences could affect the way collagen gets mineralized.

In order to characterize the rat bones subjected to immobilization, male Sprague-Dawley rats were subjected to one hindlimb immobilization using an elastic bandage. The effects of Cl2MBP treatment on bone mechanical properties using this model were reported in the last progress report. In the case of compact bone collagen,

deH-DHLNL showed a significant increase (about 30%) in immobilized bone and a tendency of an increase in pyridinoline cross-link when compared to those of control. These differences were more pronounced in cancellous bone collagen. In immobilized bone, >2-fold increase in deH-DHLNL and 50-70% increase in deH-HLNL were observed. Pyridinoline cross-links (both pyridinoline and deoxypyridinoline), in contrast to cortical bone, also increased in cancellous bone by two-fold. These preliminary data indicate that cancellous bone responds to immobilization more significantly due to its higher remodeling rate. We will further characterize these bones during the next grant period.

Based on the stoichiometry and stereochemistry of intermolecular cross-linking, we have also shown that type I collagen fibrils have more than one molecular packing modality. Since the intermolecular cross-linking is a major determinant of physicomechanical properties of the matrix, these studies could provide an explanation of amazingly diverse functions of connective tissues.

We have also been studying cross-linking chemistries in various pathological bones obtained from osteopetrotic rats, osteogenesis imperfect mice, vitamin B6-deficient chickens, and fibrous dysplasia humans. These comparative characterizations could provide data concerning possible mechanisms of the disordered mineralization.

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 and mechanisms of bone mineralization and bone loss to a clinical application.

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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 1996 Funding: $ 190,594
Students Funded Under Research: 14

Task Description:

Task 1) Human Visual Orientation (C.M. Oman)
The goal of this project is to better understand how visual scene content influences perception of orientation ("tilt", "location", "direction") and motion ("speed" and "rotation") and conversely, how the perceived orientation of objects influences perceived self-orientation in both real and virtual environments.

Task 2) Subjective Responses to Linear Acceleration and Haptic Stimulation (L.R. Young)
The overall objective of project two 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 proprioceptive) cues, mental sets, learning and experience, and active versus passive control.

Task 3) Human Factors and Physiological Effects of Short-Arm Centrifugation
We are interested in research to determine appropriate methods of applying artificial gravity to astronauts. Before a short-arm centrifuge or spinning vehicle can be tested in space, ground studies must be conducted to determine the physiological effects of a force gradient and the human factors issues associated with rotation. Thus, our project has two components: human factors and gravitational physiology. The purpose of the human factors portion of the study is to determine if the performance of two-handed tasks is affected by Coriolis forces. The second portion of this investigation focuses on determining several of the cardiovascular effects of a force gradient on a normal human subject. Studies are conducted through the use of the MIT-Artificial Gravity Simulator (AGS), essentially a two m-radius, rotating platform, in the MIT Man-Vehicle Laboratory.

For Project 1, our prototype helmet mounted Virtual Environment display system was completed, and used in three different experiments. The first was a study of tilt, vection, and visual reorientation illusions experienced by subjects inside a virtual room which slowly tumbles about them. The visual reorientation illusions have a similar character to those experienced by astronauts. Latency and saturation of vection depended strongly on scene content and symmetry, but only weakly on posture. Reorientation illusions occurred at consistent phase angles which differed between subjects. The second, not yet completed, studies the latency and magnitude of linear vection produced by a looming virtual stimulus. Independent variables include field of view, stimulus speed, orientation with respect to gravity, and adaptation/learning effects. The third is a study of the interaction of tactile, visual, and linear acceleration cues, described under Project 2 below. Script driven VR experiment

control software originally developed for this project has evolved into an Experiment Manager now being used in the NASA JSC Virtual Environment Generator on the Neurolab Shuttle Mission.

For project 2, three studies were conducted. The first study used the sled facility to investigate the hypothesis that otolith information may be used for recreating a path in space. Subjects were passively accelerated along a horizontal track and instructed to indicate when they passed a target. Three orientations were used: Y axis (interaural), +Z axis (rostro-caudal, headward), and -Z axis (rostro-caudal, footward). We found that responses were significantly more anticipatory for footward acceleration than for headward acceleration.

The second study investigated the effect of adaptation to weightlessness on ocular counter-rolling (OCR) induced by static tilt. OCR was measured pre- and post-flight in 4 astronauts on the SLS-2 mission. We found that in three of the four subjects, the magnitude of OCR was reduced post-flight. This finding lends support to the otolith tilt-translation reinterpretation hypothesis. In addition, all four subjects demonstrated changes in the left-right symmetry of OCR following flight. These results bear on both neurovestibular compensation and space motion sickness.

The third study investigated the role of tactile information in linear motion perception, using the sled facility and the NASA Langley G-seat. The G-seat was modified to permit accurate and rapid control of the pattern of tactile cues applied through the seat pan, providing a general laboratory tool. Subjects were given closed loop control of the sled, while being presented with z-axis motion stimuli in the form of sled motion, visual field motion in a helmet mounted display, and tactile cueing from the G-seat. Their task was to null out a sled velocity disturbance. Estimator transfer functions for visual, vestibular, and tactile channels were computed. The vestibular transfer function was consistent with internal integration of acceleration, and the visual transfer function indicated that the visual display velocity was interpreted as sled velocity. The tactile transfer function showed high frequency magnitudes equal to or greater than low frequency magnitudes. The high degree of variability in tactile and visual transfer functions indicates a need for further study and possibly hardware modifications.

Two studies were conducted in Project 3. In the human factors study, we questioned whether any decrease in performance of independent two-handed tasks occurs in a rotating environment and whether performance can be improved through practice and adaptation to the rotating environment. Performance was compared among three testing days which were spaced three and five days apart, to determine if any learned process carried over to subsequent days. Six male and six female subjects were rotated clockwise at 20 rpm. Subjects performed a modified Stromberg Dexterity Test in which they simultaneously exchanged the position of two blocks at a time using both hands. For this rotation level, no significant difference in steady-state performance between the prerotation, perrotation, and postrotation conditions was found. However, each time the rotation started or stopped, an initial performance decrement appeared. A more careful analysis of the data showed Coriolis forces do affect the performance of two-handed tasks, but adaptation occurs very quickly.

For the gravitational physiology study, heart rate, blood pressure, ECG signals, calf volume, and calf impedance were recorded from four men and four women rotated on the AGS. Heart rate variability was determined from the EKG signals. Trials consisted of a 30-minute rest period, 1 hour of rotation, and a final 30-minute rest period. Each subject participated in three trials such that the G level at the feet was 0.5 g, 1.0 g, and 1.5 g. An additional control trial for each subject involved 1 hour of rest, 30 minutes of standing, and a final 30-minute rest period. Post-trial analysis assessed the relationship between rotation time, G level, and the cardiovascular parameters measured. The roles gender and body morphology contributed to the cardiovascular changes were also examined. Heart rate was found to be elevated and the pulse pressure reduced at the higher G levels. The data also indicate that the major changes in hemodynamics in the peripheral appendages occur during the initial period of rotation. The results demonstrate that short-arm centrifugation can provide cardiovascular stimulation to counteract the lack of gravity in space. The cardiovascular responses to the 100% gravity gradient rotation with an acceleration of 1 g at the feet do not accurately mimic those found in standing erect, but responses to acceleration levels of 1.5 g at the feet are much closer to responses obtained when standing erect. This physiological study will be presented at the annual meeting of the International Society of Gravitational

Physiology in April 1997. Determining how a force gradient affects the cardiovascular system will enable future researchers to more precisely outline centrifuge studies necessary on individuals undergoing bedrest treatments as models for spaceflight deconditioning.

This project has a great deal to contribute to the neuro-otology community. The otolith organs are gravity sensors and are uniquely affected by the weightlessness of orbital flight. The sorts of problems created for astronauts and the ways of dealing with neurovestibular adaptation to space flight and return to Earth are highly relevant to neuro-otology and otolaryngology. The ability to "turn off" the constant pull of gravity in space provides the vestibular physiologist with an ideal tool for study of the basic function of the vestibular apparatus in spatial orientation and motor control. The process of overcoming the disturbing disorientation and space sickness associated with space travel should bear on the process of vestibular rehabilitation of patients on Earth.

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Growth Regulation in the Adult Cardiac Muscle Cell

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Funding:

Project Identification: 199-08-17-72/P01HL48788
Solicitation: RFA
Initial Funding Date: 8/93
Expiration: 7/98
FY 1996 Funding: $74,996
Students Funded Under Research: 0
Joint Agency Participation: NIH/National Heart Lung and Blood Institute

Task Description:

This grant is based on the hypothesis that normal cardiac structure, composition, and function are the direct and dynamic ongoing result of a normal myocardial loading environment, not a fixed property of cardiac tissue. Deviations above or below this normal loading set point cause abnormalities in each of these myocardial properties. This hypothesis applies equally to increases and decreases in cardiac load. Astronauts exposed to microgravity (decreased load) for greater than seven days develop a decrease in cardiac mass (atrophy). Patients with long-standing pressure or volume overload (increased load) develop an increase in cardiac mass (hypertrophy). Whether mechanisms which control an increase in mass are equal and opposite to those which control a decrease in mass is unknown; however, it is likely that insights into one process will aid understanding of both of the possible mechanisms causing alterations in cardiac mass include a change in myocardial load or a change in neurohumoral activation. Importantly, these two potential mechanisms may be complementary rather than alternative. Attempts to examine these mechanisms have been limited by the complexities of *in vivo* experiments, where it is difficult to completely separate changes in load from changes in neurohormones. An alternate approach would be to study growth regulation in primary cell culture. To date, however, methods have not been developed which allow adult mammalian cardiac muscle cells (cardiocytes) to be maintained in long-term primary culture in mitogen-free medium, with no extensive changes in phenotype and with preserved mechanical and electrical function. Thus, the specific aims of this grant are to: 1) develop methods by which adult cardiocytes can be maintained in long-term culture and be induced by alterations in mechanical load to have a graded increase or decrease in cell mass; 2) determine the relative importance of load changes versus changes in neurohormones in altering growth regulation; and 3) determine the mechanisms by which load alterations are transduced into changes in cell mass. Preliminary studies suggested that cardiocyte mass could be decreased, increased, or maintained unchanged in long-term culture using electric field stimulated contraction and specific culture methods. These changes in cardiocyte mass appeared coordinate with changes in protein synthesis rate. Based on these studies, we designed protocols in which cardiocytes will be embedded in an agarose matrix, perfused with medium, electrically stimulated to contract, and maintained in culture for 7-14 days. Graded alterations in the major determinants of load: stimulation frequency, tension development, cardiocyte length, and the tension-time index will be imposed on cardiocytes in long-term culture. As the percent agarose is increased, the matrix becomes stiffer, cardiocyte contraction becomes more nearly isometric, and cardiocytes develop more tension. As the agarose is stretched, cells embedded in the agarose will be stretched to longer cell lengths. Sequential effects of these protocols on cardiocyte morphology, function, mass, and protein synthesis, and the

mechanisms affecting these changes in growth regulation will be examined. These studies will define the primary dynamic regulators of the structural and functional properties of adult myocardium.

Specific Aim #1: Develop methods to maintain adult cardiac muscle cells in long-term primary culture with preserved normal phenotype and normal contractile function. This specific aim has been partially completed. We developed a method to maintain adult cardiac muscle cells in long-term primary culture using a specific and well-defined media with cells cultured on laminin multiwell trays. Using this model cardiocytes maintain normal phenotype and normal contractile function. These methods are described in a manuscript entitled: Growth Effects of Electrically Stimulated Contraction on Adult Feline Cardiocytes in Primary Culture, *American Physiology,* 1995, 268: H2495 - H2504. We have begun to culture adult cardiocytes in a matrix using either agarose or type I collagen. We have performed short term studies which examined protein synthesis rates in cells embedded for 24 hours and electrically stimulated. Culture for longer term will require development of methods which allow adequate diffusion of buffer through the gel in volumes large enough to maintain normal electrolyte and nutrient levels.

Specific Aim #2: Develop methods to induce a graded decrease or increase in cardiac muscle cell mass by imposing an alteration and mechanical load on adult cardiac muscle cells maintained in primary culture. Determine the effects of these changes on cell mass and cardiocyte mechanical function. This specific aim has been partially completed. Using the models outlined under Specific Aim #1 combined with electrical stimulation, cardiac muscle cell mass increased over a 7 day period of time in part because there was a significant increase in protein synthesis rate. These methods are also described in the manuscript cited above.

Specific Aim #3: Determine whether alterations in cell load (stress) or cell length (strain) contribute to changes in muscle cell mass. We have begun looking at the effects of changes in cell load (stress) by embedding cardiocytes in variable concentrations of type I collagen. To date we have done preliminary studies showing that protein synthesis rate increases in parallel with an increase in the percent collagen used to make the gel from 1% - 16%. As the percentage of collagen in the gel is increased, the gel stiffness increases and the impediment to shortening increases causing the cardiocytes to contract more isometrically and with more force. The increasing impediment to shortening has been documented using an edge detection system to measure cell shortening extent and velocity. To prove that this decrease in shortening was caused by an increased load rather then a nonspecific injury to cells, contraction was examined in cells embedded in collagen and then in the same cells after the collagen matrix was removed by treatment with collagenase. After removal of collagen, contraction returned to normal.

Specific Aim #4: Determine whether neurohormones of the sympathetic nervous system or the renin angiotensin aldosterone system alter growth regulation in adult cardiac muscle cells. Using the model outlined under specific aim #1, we examined the effect of angiotensin II on protein synthesis rate and cardiac muscle cell growth during seven days of culture with and without electrical stimulation. Angiotensin II caused a moderate growth effect but did not augment growth in the presence of electrical stimulation. In addition, angiotensin AT1 receptor blockade with Losartin or AT2 receptor blockade with PD123319 did not inhibit the growth effect of electrical stimulation. In these studies angiotensin II binding studies were performed to examine the affinity and number of angiotensin II receptors in each of the above protocols. None of these protocols affected angiotensin II binding. These experiments are described in a manuscript entitled: Comparative effects of contraction and angiotensin II on growth of adult feline cardiocytes in primary culture," *American Physiology*, 1996, in press.

Studies examining the Specific Aim #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.

FY96 Publications, Presentations, and Other Accomplishments:

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Mechanical and Molecular Stimuli for Normalizing Muscle Mass During Unloading

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Funding:

Project Identification: 199-40-17-04
Initial Funding Date: 2/95
FY 1996 Funding: $ 156,725

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 4

Task Description:

Unloading of postural skeletal muscle, as seen during space flight or ground based models such as bedrest or limb suspension, may result in loss of muscle mass and changes in myosin heavy chain (MHC) phenotype. A series of studies are proposed that would more clearly define the primary mechanical stimuli required for altering fiber size and inducing transformations in MHC phenotype. The proposed studies will impose precisely controlled contractile training paradigms ranging from protocols which optimize power output to those which result in near maximal force production on single muscles in the unloaded (via tail suspension) hindlimbs of rats. These training paradigms will be evaluated with respect to various parameters (e.g., mass, MHC type, total protein and RNA content) to identify the best candidates for an exercise countermeasures program. To investigate the involvement of insulin like growth factor 1 (IGF-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.

Research Aim - Elucidation of the role of IGF-1 in the maintenance of muscle mass.

Recent results (*J. Appl. Physiol.* 81(6):2509-2516, 1996) of studies funded by this grant have provided information in two areas critical to our understanding of the role of IGF-1 in the maintenance of muscle mass. These results clearly indicate that increases in IGF-1 precede the hypertrophy response and thus have the appropriate temporal relationship to be considered a mediator of this process. Second, changes in IGF-1 are correlated with changes in total muscle DNA. This finding lays the groundwork for a mechanism for IGF-1 mediated support of the hypertrophy process. Our data lead to the hypothesis that muscle overloading stimulates the production of IGF-1 by muscle cells leading to anabolic processes and to the activation and incorporation of satellite cells which allows the continuation of the hypertrophy process. To further elucidate the role of IGF-1 in the maintenance of muscle mass we have developed a method for the direct delivery of non-systemic doses of IGF-1 directly into single target muscles. Initial results show that direct infusion of IGF-1 can result in hypertrophy of the target muscle with no observed effects on adjacent muscles or other tissues (e.g., heart). IGF-1 infusion for 3 weeks increased muscle DNA levels in a manner similar to that reported above during muscle hypertrophy.

Research Aim: Identification of the Optimal Mechanical Stimulus for Muscle Mass Sparing:

To accomplish this research aim the soleus muscles of rats are chronically instrumented with electrodes to permit electrical stimulation when the rats are mounted on a training platform attached to a computer controlled ergometer. The hindlimbs of these rats are unweighted via tail suspension in special cages and their soleus muscles are trained at regular intervals. To date, two isometric training regimens have been shown to be effective in preventing most of the atrophy seen in soleus muscles of hindlimb suspended rats.

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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Funding:

Project Identification: 199-40-17-06
Solicitation: 12-10&11-92/GB
Initial Funding Date: 1/95
Expiration: 12/95
FY 1996 Funding: $0
Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

1) *In vitro* studies of cell proliferation and hair cell regeneration

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 *with* and *without* otolith membranes in amphibian culture medium (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 condition of our cultures with vital fluorescent dyes and their morphological integrity with differential interference contrast (DIC) optics. Saccular and utricular cultures were maintained with minimal cell damage for up to 7 and 11 days, respectively. 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. Within this time frame, the otolith membranes of organs without this structure were only marginally restored, allowing good visibility of hair cells and their sensory hair bundles.

Organ cultures pre-incubated for 6 hrs in culture medium supplemented with 350 μM gentamicin sulfate displayed extensive hair bundle and cellular damage by 2 days after gentamicin treatment. This damage, as *in vivo,* was seen throughout the saccular macula and 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, as *in vivo,* 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 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% of its normal value by 7 days.

To determine if hair cell regeneration could take place in the absence of cell proliferation, we cultured normal and gentamicin-treated organs in culture medium supplemented with 25 μm 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 implying that early hair cell recovery was largely due to non-mitotic mechanisms.

(2) Immunocytochemical studies of cytoskeletal and calcium-binding proteins

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, were 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.

(3) Immunocytochemical studies of myosin in saccular and utricular hair cells

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.

(4) Dye-labeling studies of hair cells and supporting cells

We have used intra- and extracellular labeling techniques to dye-label mature hair cells and supporting cells in wholemount saccular cultures. These dye-labeled cells have resting potentials and input resistances similar to hair cells and supporting cells in acute preparations and these cultures, as in our earlier studies, exhibit both cell proliferation and hair cell regeneration. During the past year, we have successfully cultured wholemount saccular maculae with *mature*, *immature*, and (in the saccular margins) *developing* dye-labeled hair cells in sealed culture chambers under both normal and mitotically-blocked conditions. We have also verified that dye-labeled hair cells can be observed under low-light conditions without inducing photobleaching or compromising culture conditions.

In parallel studies, we have also been intracellularly labeling single hair cells with Lucifer Yellow and extracellularly labeling these hair cells and their surrounding supporting cells with carbocyanine or Cell Tracker dyes which selectively label the plasma membrane and cytoplasm, respectively, of living cells. This double-labeling procedure, in conjunction with fluorescent time-lapse microscopy, allows us to study the extrusion of damaged hair cells and the mechanisms of scar formation and repair in supporting cells following ototoxic treatment. Most excitingly, we have recently demonstrated that we can intracellularly dye-label both *normal supporting cells and supporting cells participating in scar formations* in wholemount organ cultures. Using these techniques, we are now determining if supporting cells in wholemount organ cultures acquire hair cell-like characteristics and studying the *in vitro* morphological and physiological development of immature hair cells.

(5) Whole-cell patch clamp recordings from normal and regenerating hair cells

Our *in vivo* and *in vitro* studies of hair cell regeneration in the bullfrog vestibular otolith organs following aminoglycoside ototoxicity 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 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.

Our preliminary experiments have characterized the passive membrane properties of hair cells and supporting cells in normal and gentamicin-treated saccular cultures. We have also studied the whole-cell membrane current of normal hair cells and supporting cells. We are now examining the biophysical properties of supporting cells with hair cell-like characteristics and regenerating hair cells isolated from gentamicin-treated cultures to compare the whole-cell membrane currents in these cells with those seen in normal hair cells and supporting cells. We are also studying if the complement or the kinetic properties of individual conductances in new hair cells is altered during the regeneration process.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Baird, R.A., Torres, M.A., and Schuff, N.R. Ongoing and gentamicin-induced hair cell regeneration in the bullfrog vestibular otolith organs. I. Relationship to cell proliferation. J. Comp. Neurol, (in press).

Baird, R.A. and Schuff, N.R. Ongoing and gentamicin-induced hair cell regeneration in the bullfrog vestibular otolith organs. II. Contribution of non-mitotic mechanisms. J. Comp. Neurol, (in press).

Baird, R.A., Bale, S., Fiorillo, C., and Schuff, N.R (abstract) *In vivo* and *in vitro* evidence for non-mitotic hair cell regeneration of vestibular otolith hair cells. Assoc. Res. Otolaryngol. Abstr, 18, 45 (1995).

Baird, R.A., Bales, S., Fiorillo, C., and Schuff, N.R. Hair cell regeneration in the bullfrog vestibular otolith organs does not require cell proliferation. Auditory Neurosci., (in press).

Baird, R.A., Steyger, P., and Schuff, N.R. Cell proliferation and hair cell regeneration in the bullfrog vestibular otolith organs. N.Y. Acad. Sci., 781, 59-70 (1996).

Hawkins, J.R., Steyger, P.S., Burton, M.D., and Baird, R.A. (abstract) Calcium-binding proteins may be early markers of non-mitotically regenerating vestibular otolith hair cells. Assoc. Res. Otolaryngol., 19, 7 (1996).

Steyger, P.S., Dumont, R.A., Gillespie, P.G., and Baird, R.A. (abstract) Myosin Ib immunolabeling in hair bundles of adapting and non-adapting vestibular hair cells. Assoc. Res. Otolaryngol., 19, 186 (1996).

Effect of Skeletal Unloading on Bone Formation

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Project Identification: 199-40-47-01
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $ 176,242
Students Funded Under Research: 1

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.

The major effort in 1996 was to develop cellular models to evaluate the effect of skeletal unloading on bone formation. The first set of experiments employed cells obtained from loaded and unloaded bones and evaluated in culture under comparable conditions. The second set of experiments employed cells from normally loaded rat bones but studied in culture with or without mechanical strain using a Flexercell. Concurrent with this effort we evaluated the ability of parathyroid hormone to reverse the ability of skeletal unloading to inhibit bone formation.

For the first set of experiments osteoprogenitor cells were obtained from hind limb elevated (2 and 5d) and control rat tibiae, grown in culture for up to 28 days and evaluated for mRNA levels of c-fos and alkaline phosphatase. The mRNA levels were determined using competitive PCR, a technique which we developed by incorporating a 25bp piece of DNA into a convenient restriction site of each gene that we were interested in assessing and using this "competitor" in the PCR assay for the mRNA of interest. As expected c-fos mRNA rose early in culture reaching a peak between days 5-10, whereas alkaline phosphatase mRNA levels peaked around day 15. Hindlimb elevation resulted in a decrease in peak c-fos and alkaline phosphatase mRNA, and a delay in the subsequent rate at which the mRNA levels declined. The decrease in alkaline phosphatase mRNA was accompanied by a decrease in alkaline phosphatase activity and, ultimately, a decrease in calcified nodule formation. The experiment is currently being repeated to measure c-fos, alkaline phosphatase, osteocalcin, IGF-1 and IGF-1 receptor mRNA, and to evaluate whether IGF-1 can alter the differential expression of these genes. These data have been submitted for publication.

We then evaluated the ability of mechanical strain to modulate the effect of IGF-1 on cell growth and differentiation. Our experiments to date remain preliminary. However, exposing the cells to 8h of cyclic 2% strain for 4d stimulated alkaline phosphatase activity additive or synergistic to that of 10ng/ml IGF-1. These experiments are continuing to optimize the parameters of strain.

We have completed a study examining the influence of hindlimb elevation on PTH stimulated bone formation. The results indicate that the ability of PTH to stimulate bone formation is site specific with greatest stimulation in the distal periosteum (TFJ) and secondary spongiosa of the proximal tibia; the distal endosteum and proximal periosteum of the tibia is less responsive. Hindlimb elevation does not block PTH action, although some reduction is observed. These data have been accepted for publication. These results are consistent with our *in situ* hybridization and immunolocalization results localizing the PTH receptor, and demonstrating no major effect of hindlimb elevation on the PTH receptor. These data are being prepared for pubication. We are completing our studies examining the effect of PTH injections on bone cell proliferation *in vivo* using BrdU labeling. Our results indicate that PTH does not stimulate proliferation.

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.

FY96 Publications, Presentations, and Other Accomplishments:

"Hormonal Regulation of Bone Mineral Metabolism." Edited by: Bikle, D.D. and Negro-Villar, A. Endocrine Society Press, Bethesda, MD, 1995.

Bikle, D.D. "Vitamin D: New actions, new analogs, new therapeutic potential" in "Hormonal Regulation of Bone Mineral Metabolism." Edited by: Bikle, D.D. and Negro-Villar, A. Endocrine Society Press, Bethesda, MD, pp 77-83, 1995.

Bikle, D.D. Vitamin D: A bright future for the sunshine hormone. Sci. Amer. Sci. & Med., March/April, 58-67 (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 1996 Funding: $0
Students Funded Under Research: 0

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

Results from FY 95 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.

Information regarding specific progress made during FY96 was not provived by the principal investigator.

Otolith-Canal Convergence in Vestibular Nuclei Neurons

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-40-17-03
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $57,467
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 nerve fibers innervating the peripheral receptors of the semicircular canal and otolith system in birds. The anatomical coordinates of the major planes of the semicircular canals were determined as referenced to their orientations in the skull. These planes represent the best stimulus direction for rotational head motion as theoretically predicted from fluid mechanics. It was found that the planes of the three semicircular canals have significant deviations from orthogonality relative to each other. Next, the responses of primary afferent fibers innervating each of the three semicircular canals were obtained to different directions of rotational head motion. The best directional response was determined for each afferent. It was found that for the horizontal and posterior semicircular canal afferents, good agreement between the anatomical canal planes and the afferent response vectors were obtained. However, for the anterior semicircular canals, afferents were found to synthesize the information from the different anatomical planes of the canal. The result showed that canal afferents preserved orthogonal directional information regarding rotational head movements, even though the anatomical canal planes have significant deviations from orthogonality. Next, the directional tuning of otolith afferents in pigeons were determined using linear acceleration stimuli. The goal was to determine how otolith afferents encode directional motion relative to gravity. The responses of otolith afferents 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 showed that most utricular fibers 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 ultricular 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 10Hz (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 spaceflight, 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.

FY96 Publications, Presentations, and Other Accomplishments:

Dickman, J.D. Spatial orientation of the semicircular canals and their innervating afferents in pigeons. Exp. Brain Res., 111, 8-20 (1996).

Dickman, J.D. Vestibular afferent projections to the brain stem in pigeons. Ann. N.Y. Acad. Sciences, New Directions Vest. Res., 781, 611-613 (1996).

Dickman, J.D. and Fang, Q. Differential central projections of vestibular afferents in pigeons. J. Comp. Neurol., 367, 110-131 (1996).

Si, X., Angelaki, D.E., and Dickman, J.D. Response properties of pigeon otolith afferents to linear acceleration. Association for Research in Otolaryngology Abstracts, St. Petersburg, FL, (February, 1996).

Microgravity In Vitro *Model of Bone Cells: Flow Effects*

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Funding:

Project Identification: 199-40-27-12
Solicitation:

Initial Funding Date: 3/95
Expiration: 2/97

FY 1996 Funding: $0
Students Funded Under Research: 3

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 bedrest 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 remodeling *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 remodeling 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 mg/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.

FY96 Publications, Presentations, and Other Accomplishments:

Hillsley, M.V., and Frangos, J.A. Osteoblast hydraulic conductivity is regulated by calcitonin and parathyroid hormone. J. Bone & Min. Res., 11, 114-124 (1996).

Johnson, D.L., McAllister, T.N., and Frangos, J.A. Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts. Am. J. Physiol., 271, E205-E208 (1996).

"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 1996 Funding: $98,570
Students Funded Under Research: 2

Task Description:

A newly-developed culture system for mammalian cells, called the "baby machine," has properties ideally suited for studies on the direct effects of gravity on cell growth and division. The advantage of the system is that the cells can be oriented with respect to the gravity vector in the absence of additional external constraints such as the cell-substratum and cell-cell interactions. This culture system will enable ground-based assessments of gravity-sensitive "windows" for any cell process. In this proposal, gravitational effects on mitosis, the cell cycle, the segregation of components between daughter cells, and cellular senescence will be evaluated. Growth and division of the cell cultures will be analyzed with respect to fixed gravity vectors, and during gravity averaging in a clinostat. The involvement of the gravity vector in the orientation of mitosis will be determined, as well as the existence of gravity-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. In the baby machine culture system, cells are fixed in place so that they can be positioned precisely with respect to a fixed plane, thereby enabling ground-based assessments of gravity-sensitive "windows" for any cell process. The aims for FY96 were to quantitate the effects of the gravity vector on the orientation and frequency of cell division. For this purpose, the bottoms of 25 ml polystyrene culture flasks were first coated with a 5% solution of polyhydroxyethyl methacrylate (polyHEMA). Cells do not adhere to polyHEMA. Next the adhesive sites were introduced to the coated flasks in the form of 4.8 μm-diameter Dynabeads (Dynal, Inc.). The Dynabeads, generally the tosyl-activated version, were resuspended in 5 to 10 ml of PBS to a total of approximately 106 beads/sample. After vortexing the suspension, it was poured into a polyHEMA-coated flask, and the beads were allowed to settle onto the coating for 6 hr or longer at room temperature. CHO cells, in RPMI 1640 medium containing 10% FCS, were added to the flask and they attached only to the beads. The cells grew and divided while attached to the beads and shed one of the two new progeny. Two major conclusions were reached. First, when CHO cells were held fixed in a vertical plane, the direction of gravity had no effect on the orientation of the division plane. Since the division plane is perpendicular to the spindle axis, it appeared that the determination of spindle orientation overwhelms any possible gravitational effects, and that the randomness of division orientation reflected the randomness of the attachment of the cells to the adhesive sites. Second, the axis of cell division was either in the same plane or in a 90° plane in consecutive divisions. If spindle orientation, and thus division plane orientation, is a consequence of the movement of

centrosomes to opposite sides of the nucleus to establish the locations of the spindle ends, this movement can take place in either the same plane or an orthogonal plane in successive cell cycles. This indicates that division in somatic cells differs from the early cleavages in embryos in which this movement is invariably in a fixed sequence of orthogonal planes. This methodology is also being used to investigate the effects of gravity averaging, in a clinostat arrangement, on cell cycle times. However, in spite of success with the baby-machine technique as described here, we had not yet reached the real potential of the method — a culture system in which one of the two progeny cells is always shed from the surface, and the one that remains is always the same when the two can be differentiated. Such a technology is important for the following reasons: 1) The process would be a continuous culture device, producing newborn cells continuously for use at any time in any cell cycle study, because the number of attached cells in the culture would remain constant indefinitely; 2) The cells would be in steady-state growth, due to the continuous culture aspect, so that the cell cycle studies would be performed on undisturbed cells; 3) The process is ideal for analyzing any aspect of the partitioning of any component or developmental signal between cells at division since one progeny is always shed and the same one remains in the instrument; and finally, and perhaps most important, 4) this culture system would automatically enable identification of changes in cells as they age or senesce. Since the same site on the same cell remains attached, the attached cell must always be the older of the two in those instances when the cell ages can be distinguished. We thus began to examine the possibility of holding the cells in place indefinitely by a pressure differential. A cell is drawn to a hole in a solid surface, smaller than the cell diameter, and held in place with a mild pressure differential. When the cell divides, only the daughter associated with the hole will remain attached and the other will be released, since the pressure differential is blocked by the attached cell. During FY97 the culture system should be in routine use in our lab for analysis of gravity effects on the mitotic cycle during long-term incubation. It will also be used to investigate the effects of cellular replicative age on the sensitivity of the cell cycle and chromosome segregation to gravity and gravity averaging.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Helmstetter, C.E. Regulatory Aspects of Chromosome Replication and Cell Division. Walt Disney Memorial Cancer Institute, Orlando, October 28, 1995.

The Effects of Microgravity on Bone Osteoblast Growth

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Funding:

Project Identification: 199-40-27-06
Solicitation: 91-OSSA-15

Initial Funding Date: 1/93
Expiration: 12/96

FY 1996 Funding: $
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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-G is the loss of natural mechanical stress experienced on Earth. Mechanical stress (exercise) has been used by both the Soviet and American programs as a countermeasure for bone loss in flight. Mechanical stress has recently been demonstrated to cause release of prostaglandin E2 (PGE2) from osteoblasts. PGE2 has been shown to increase trabecular bone formation in rats and infants, but the mechanism of action of PGE2 in stimulating osteoblast growth is unknown. A second and indirect effect of microgravity is an increase in 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-G. We have developed a culture system using the MC3T3-E1 cloned osteoblast as our model to study the molecular

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

Our first objective is to study the effect of mechanical stress on prostaglandin release and osteoblast cell growth. We will study the signal transduction of prostaglandin stimulated bone growth and will analyze the gene regulation of osteoblasts under inhibited and stimulated conditions. Our second objective is to understand the role of 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-G 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 astgronauts 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 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, it was reported that the glucocorticoids are increased on a daily average in astronauts. We followed up that discovery with studies on the ground where we used comparable amounts of glucocorticoids found in astronauts and patients and published data showing that the glucocorticoids decrease new bone growth by 50%. This growth is partially to fully reversed by addition of exogenous PGE2. We have also found in our flight experiment on STS-56 that microgravity interferes with normal bone cell growth activation and causes reduced PGE2 synthesis, 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 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 spaceflight also add 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).

Spin-off benefits: 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 (1, 4, 5, 8, 9, 12, 15) and may be the basis of aspirin protection from mortality in colorectal cancer patients.

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Mechanochemical Coupling between ECM and the Cytoskeleton

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Funding:

Project Identification: 199-40-27-15
Initial Funding Date: 9/95
FY 1996 Funding: $0

Solicitation:
Expiration: 8/96
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

In the course of this project, we have demonstrated that living cells are literally "hard-wired" such that mechanical stresses applied to the cell surface are transmitted across the membrane, through the cytoplasm, and to the nucleus over discrete molecular pathways. We showed that transmembrane adhesion receptors that physically interlink with the internal actin cytoskeleton, such as integrins and selectins, provide preferential paths for transmembrane transfer of mechanical signals. We also demonstrated that signal transfer to the cytoskeleton can be modulated by altering the molecular composition of the focal adhesion complex, that is, by changing the structure of the molecular bridge that interlinks integrins with actin filaments. Importantly, we also found that many of the signaling molecules that mediate signal transduction by growth factors and extracellular matrix are also physically immobilized on the cytoskeletal backbone of the focal adhesion complex. Mechanochemical transduction may therefore be mediated by stress-induced CSK rearrangements that alter the distribution or shape (chemical potential) of associated signaling components. In the course of studying this process, we developed a magnetic twisting cytometry technique that permits us to quantitate changes in CSK

mechanics in real-time within living cells. This technique has added experimental support for our hypothesis that living cells use tensegrity architecture to organize and stabilize their CSK and hence, control cell shape and function. In addition, we have developed 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 the tensegrity mechanotransduction 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.

FY96 Publications, Presentations, and Other Accomplishments:

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Are G Proteins Mechanosensors for Endothelial Cells?

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Funding:

Project Identification: 199-40-27-21
Solicitation: 93-OLMSA-07
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $96,878
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 senses changes to mechanical perturbation such as cyclic strain may be pertinent to that which occurs in response to changes in gravity. The objective of the proposed studies is to examine the effect of mechanical signaling at the cellular level. Preliminary data demonstrate that acute cyclic strain of bovine aortic EC causes loss of immunoreactivity of the inhibitory G protein alpha subunits Gi(1,2), that correspond temporally to the activation of the adenylate cyclase signal transduction pathway. The specific hypothesis to be tested in these studies is that strain-induced loss of Gi(1,2) is caused by post-translational modification of this protein directed at the carboxyl terminus. The hypothesis is based on preliminary data that show strain-induced loss of Gi(1,2) is limited to antisera that recognize the carboxyl terminus of Gi(1,2. The carboxyl terminus of Gi(1,2) contains a CAAX motif that is a well recognized site of post-translational modifications such as prenylation, carboxymethylation, and ADP-ribosylation.

We have continued to make significant progress toward delineating the role of G proteins as mechanosensors for endothelial cells as well as smooth muscle cells. The major accomplishments are as follows:

Nicotinamide, an inhibitor of mono(ADP-ribosyl)transferase, prevents basal and strain-induced stimulation of nitric oxide gene expression in bovine aortic endothelial cells. Previous studies obtained in our laboratory suggest that cyclic strain stimulates ADP-ribosylation of G proteins, namely the inhibitory G protein, Gi(1,2), in bovine aortic endothelial cells. In addition, Awolesi et al. (*J. Clin. Inv.* 96:1449,1995) showed that cyclic strain also stimulates nitric oxide gene expression. Based on these findings and earlier work by Kuchan et al. (*Am. J. Physiol.* 267:C753,1994) that demonstrated G protein involvement in shear-activated nitric oxide production, we tested the hypothesis that blockade of strain-activated ADP-ribosylated Gi (by nicotinamide treatment) would prevent cyclic strain stimulation of nitric oxide gene expression. Exposure of endothelial cells to nicotinamide for 3 hours prior to the initiation of cyclic strain, and for the duration of a 24 hour cyclic strain regimen of 150 mmHg at 60 cycles/min, prevented both basal and strain-induced nitric oxide production. These data suggest that ADP-ribosylation of Gi may be involved in constituitive as well as cyclic strain-stimulated nitric oxide gene expression. Alternatively, the effect of nicotinamide may involve non-specific effects of the drug such as its free radical scavenger action. Thus,

follow-up experiments are warranted with other known inhibitors of G protein ADP-ribosylation such as Vitamin K1. In addition, we also plan to examine whether blockade of ADP-ribosylation of Gi will prevent the expression of other cyclic strain responsive genes in endothelial cells (e.g., prostacyclin synthase).

G proteins may also act as mechanosensors in microvascular endothelial cells. Previous studies have shown that strain-induced activation of the adenylyl cyclase/cyclic AMP/protein kinase A pathway parallel the ADP-ribosylation of G proteins in bovine aortic endothelial cells. We wished to determine whether similar pathways are activated in endothelial cells obtained from smaller microvascular beds known to be important in the control of vascular tone. We have observed cyclic strain-induced stimulation of cyclic AMP accumulation and protein kinase A activation, respectively, in immortalized dermal microvascular endothelial cells (HMEC-1). However, unlike our previous findings in bovine aortic endothelial cells, we were unable to detect a downstream stimulation of CRE binding protein activity that may simply be attributable to the time point (60 minutes) chosen for study. Alternatively, these data may suggest alterations in the transduction profiles of endothelial cells dependent on the vascular bed derivation. Other studies demonstrate differences in the end-responses of HMEC-1 as compared to bovine aortic smooth muscle cells. For example, cyclic strain clearly stimulates proliferation in endothelial cells obtained from bovine aorta whereas the proliferative rate in HMEC-1 in response to cyclic strain is unchanged (data not shown).

3. **G proteins may act as mechanotransducers in smooth muscle cells.** Smooth muscle cells, like endothelial cells, exhibit strain-induced phenotypic changes such as increased proliferation and alignment. Thus, we also tested the hypothesis that cyclic strain activates the adenylyl cyclase/cyclic AMP/PKA pathway in smooth muscle cells. We found that with regard to this pathway, smooth muscle cells and endothelial cells derived from bovine aorta behave similarly as follows: Basal adenylate cyclase activity was elevated nearly two-fold in SMC subjected to 10% average strain for 30 min and was not significantly greater than control for 60 minutes. Cyclic AMP was also stimulated by cyclic strain at 30 minutes and returned to basal levels by 60 minutes (data not shown). The stimulation of this pathway is strain specific since SMC selectively seeded on either the periphery or center of membranes subjected to 150 mm Hg for 30 minutes demonstrated significantly ($p<0.05$) higher PKA in the center (0-7% strain) but not in the periphery (7-24% strain). Likewise, CRE binding protein levels were elevated in SMC seeded in the center but not in the periphery.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Skeletal Collagen Turnover by the Osteoblast

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Funding:

Project Identification: 199-40-47-02
Initial Funding Date: 3/95
FY 1996 Funding: $94,766

Solicitation: 93-OLMSA-07
Expiration: 2/98
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. We have determined that there is minimal transcription of the collagenase gene in proliferating osteoblasts, and that the gene becomes transcriptionally active when the cells are mineralized and differentiated. This was done by the method of nuclear run-on assays. Thus, the transcriptional rate is the determinant of changes in mRNA abundance. Now that we have this information, we have returned to the transfection experiments.

From other work with the rat osteosarcoma line, UMR 106-01, we have determined that the elements in the rat collagenase gene responsible for PTH regulating transcriptional activity are the AP-1 site, a basal element, and a *runt* domain (RD) binding site upstream of the AP-1 site, which appears to be an osteoblast-specific element, in many bone genes. We have now done transfections of promoter constructs with each of these elements. In the proliferating cells, promoter activity is very low, correlating with the mRNA abundance and rate of transcription experiments for the endogenous gene. We are completing the difficult experiments with the mineralizing cells. After many different approaches to transfecting non-proliferating, mineralized cells we found that the original approach was still the best, using calcium phosphate co-precipitation. The preliminary data suggest that the two elements identified to be the PTH-responsive elements are also responsible for regulation of differentiated expression of the collagenase gene in normal osteoblasts.

Concurrently, we are obtaining information as to what proteins bind to these two elements in the normal differentiating osteoblasts. Two approaches are being taken to do this. The first is the gel mobility shift assay, which determines whether proteins will bind to a specific sequence of DNA. Using the RD binding site sequence, we have found that there is a novel shifted band in the mineralizing cells compared with the proliferating cells. We also know that the proteins binding to this element are members of the acute myelogenous leukemia (AML) family of human transcription factors. We are also undertaking experiments to

assess the expression of members of this family by Western and Northern blots. Once we have more information on the proteins binding here, we will start the process of cloning them. We are also examining the AP-1 members binding to the AP-1 site.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Hyper-G Studies of Vestibular Maculas Neural Plasticity

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Funding:

Project Identification: 199-40-12-01
Initial Funding Date: 2/95
FY 1996 Funding: $132,000

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 0

Responsible NASA Center: ARC

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 four 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 one and, once complete, correlated anatomical and physiological studies will begin. Rats will be centrifuged for four 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 o1-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 four 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.

During FY96 sufficient data were collected to conclude statistical analysis of synaptic ribbon changes in hair cells of gravity sensors of rats exposed to microgravity on the SLS-2 mission. More than 1,000 hair cells and over 6,500 synaptic ribbons were studied in 12 samples. In all, synaptic ribbons in 1,200 serial sections were recorded. The results have been written into a report for publication in FY97. The main finding is that type II cells are particularly affected by space flight. Type II hair cells may be detectors of gravity and type I cells detectors of transient translational linear accelerations. In addition, 3-D reconstructions were made from the serial sections to illustrate particular facts about the neuronal connections in gravity sensors. The

reconstructions showed that type II hair cells are arranged in small clusters, typically of three cells, with the surrounding calyces providing overlapping innervations to the type II cells. In one reconstruction, five calyces and one afferent nerve fiber together provided 18 processes to one type II cell. The findings continue to support the concept that type II cells are integrated into the same neuronal circuitry that supplies type I cells. However, the ultrastructural research also showed that type II cells receive presynaptic processes of afferents as well as postsynaptic processes, and that some synaptic interactions are reciprocal. This result has led to the concept that there are local microcircuits in gravity sensors, and that type II cells are inserted into these microcircuits. This fact can help account for the finding that type II hair cells are particularly affected by spaceflight, since local circuits help shape a response in other systems. In this case, gravity-sensitive cells would be interacting with the output of type I cells to shape the neuronal responses to transient linear accelerations. These new insights provide a basis for research into the molecular events underlying synaptogenesis and deletion in gravity sensors. They also give new emphasis to the need to study the development of these interesting endorgans in microgravity on the space station to learn whether neuronal connectivities will be altered to such an extent during development that readaptation to Earth's 1-G will be problematical.

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.

FY96 Publications, Presentations, and Other Accomplishments:

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Ross, M.D. (invited speaker) Future trends in research and funding at NASA. Universities, research and commercial science and technology: Pursuing a competitiveness agenda. University of Arizona, Tucson, AZ. Feb. 29-March 2, 1996.

Ross, M.D. (invited speaker) The information revolution - Bridging the gap. Harvard Business School, 1996 Global Alumni Conference, San Francisco, CA. March 20, 1996.

Ross, M.D. (invited speaker) The role of biocomputation and computer-based technology in medicine in space and on earth. Medical Applications of Space Life Science Research and Technology. Aerospace Medical Association Scientific Meeting, Atlanta, GA. May 5-9, 1996.

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Transgenic Markers of Bone Cell Lineage Progression

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Project Identification: 199-40-27-14
Solicitation: 95-OLMSA-01
Initial Funding Date: 07/1/96
Expiration: 06/30/97
FY 1996 Funding: $ 133,056
Students Funded Under Research: 2

Task Description:

Bones sense the load they are required to bear and alter their architecture to compensate, either increasing strength when mechanically loaded or losing strength when unloaded. One of the key events in the remodeling process is the appearance bone-forming osteoblasts at the sites where new bone needs to be made. These cells are thought to arise from a pleuripotential stem cell which proliferates and differentiates through a number of steps into a mature bone-forming osteoblast. We have developed a family of collagen-promoter transgenes which appear to be activated at different stages in the bone cell lineage. When expressed in transgenic mice, these can serve as convenient and powerful markers to assess the process of recruitment in intact mice exposed to physiological stimuli of skeletal loading or unloading. This grant will develop a mouse model that will be useful to follow the cellular response to gravitational changes on the skeleton. The activation of these transgenes will be assessed by standard and immunohistomorphology of bone (by Dr. Yeh) and in marrow stromal cells (Dr. Rowe) derived from mice subjected to a mechanical loading (treadmill) or unloading (swimming). If these transgenes are shown to accurately reflect the cellular activities, then the model could be used to evaluate strategies for preventing bone loss during prolonged space flight by measuring transgene signals that are secreted into the blood so that the temporal response can be monitored without having to sacrifice the animal.

The first year of this new grant has forced us to come to the reality that standard methods for mechanically loading bone that have been successful in the rat do not appear to work for the mouse. We have performed a number of treadmill exercise protocols that vary the total exercise duration (three days to 30 days), length of exercise session (30 minutes to two hours a day) and angle of treadmill incline (zero-four degrees) in an effort demonstrate an exercise effect relative to control mice. It is very clear that at the level of histomorphometry, mRNA hybridization and CAT transgene activity, there is a not a significant response to these exercise protocols. We believe that the reason for this disappointing result is that the control animals exercise as much as the test mice, but they do it at night in their cage. A Finnish research group engineered a computer system to monitor the activity of a squirrel wheel placed in a mouse cage. They showed that mice can run as much as seven kilometers a night. Thus the control mice exercise through the night and can not be used as a adequate control for exercise. In retrospect, we might have anticipated this problem in a burrowing animal. We have tried to alter this behavior by altering the light cycle, placing soft bedding in the cage, and placing food on the floor of the cage without success.

To solve this problem, we are going to redefine our control population. All study mice will be adapted to long-term swimming over a two-week period. Preliminary experience shows that the mouse can swim and float in the water for six-eight hours a day without any stress. After prolonged water adaptation, the exercise group will be removed and begun on the treadmill exercise program without further swimming. The remainder of the group will continue swimming. We hope that in this way we will show a response to mechanical loading in the group that was switched from inactivity to mechanical loading.

These experiments need to be carried out in Dr. Yeh's laboratory since he has the treadmill exercise equipment . We have had difficulty getting final approval from the animal care committee to perform the swimming experiments even though it is a well described intervention by a number of research groups at other institutions. In the process of dealing with the review committee, one very useful suggestion was made that we will try to implement. The chemical Liquivent or perfluoro-octal bromide is a nontoxic liquid which holds oxygen at 20x that of water and reaches a physiological pO_2 concentration. It currently is in phase three clinical trials for a variety of shock lung disorders. We will obtain this liquid from the company to determine if it could act as a water substitute. Because it has a density of 1.92, the mice will float at the surface and can even eat their feed and obtain water from the medium. They can be maintained in the medium continuously because even if they fall asleep with their heads in the medium, they will not suffocate. Thus it may be possible to truly recapitulate prolonged weightlessness in the mice using this procedure. We will try to initiate this approach as soon as possible.

In the interim, we have developed a new set of transgenes based on green florescence protein (GFP). Transgenic lines expressing wt GFP and partially humanized S65T-GFP (GLP-Life Tech) have already been produced and are currently under evaluation. New mice expressing totally humanized GFP (S65T EGFP-Clonetech) are now in production. The autofluorescent transgenes will greatly facilitate analysis of transgene activity because tissue will no longer have to be stained by immunohistochemistry. Furthermore there are number of GFP variants that excite or fluorescence at distinct wavelengths such that more than one transgene could be combined to get a more dynamic impression of the cell population that are responding to gravitation loading or unloading.

In summary the reality of performing mechanical loading studies has to be solved if we want to take advantage of the power of the genome manipulation that the mouse provides. It has been disappointing that the most physiological method for mechanical loading (treadmill exercising) was unsuccessful. However I believe that if the Liquivent media is successful, we will develop a very standardized and physiological model for the loading and unloading of bone in transgenic mice.

A model for a bone loading and unloading that can be easily standardized and applicable to transgenic mice opens a way to study the molecular and cellular controls of gravity on intact bone. Models that use cultured cells lack this essential ingredient. Other models in intact rats that use tail lift, limb restraints, or partial neurotomy are far from physiological. Interpretation of experimental results are always clouded by secondary effects that might not arise in a more physiologically relevant model. We chose treadmill exercise as the most physiological model, but have had difficulty showing an exercise effect relative to caged non-exercised mice. This result arises because the control mice spontaneously exercise in the cage.

To overcome the problem of the control, a method of prolonged unweighting of bone needs to be implemented. We believe that a model that utilizes treadmill exercising or fluid floating will most closely reassemble long-term weighting or unweighting of the skeleton. Either water or an artificially high oxygen carrying medium will be employed to provide a prolonged exposure to limb unloading. These mice will serve as controls to the mice placed under standard treadmill protocols.

New autofluorescent transgenes are being incorporated into the cell specific promoters so that bone cell lineage progression can be assessed in the transgenic mice with greater ease and discrimination. We hope that the transgenic mice that we have produced and a method to exercise or float mice can become a standardized and reproducible model so that comparison of different aspects of mechanical loading of bone can be interpreted from one laboratory to another.

FY96 Publications, Presentations, and Other Accomplishments:

Bedalov, A., Salvatori, R., Kapural, B., Kronenberg, M., Dodig, M., Clark, S.H., Woody, C.O., Rowe, D.W., and Lichtler, A. Tissue specific expression of the COL1A1 promoter in transgenic mice: Identification of 49 bp region required for transgene expression in bone. J. Bone Min. Res., 10, 1443-1451 (1995).

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Mechanosensitive Ion Channels in Bacteria

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Funding:

Project Identification: 199-40-17-07
Initial Funding Date: 12/95
FY 1996 Funding: $108,368

Solicitation: 95-OLMSA-01
Expiration: 11/98
Students Funded Under Research: 3

Task Description:

The vast majority of organisms respond to touch, vibration, gravity and changes in osmotic pressure. However, the molecular mechanisms of these phenomena remain largely unknown. Recently we have succeeded in biochemical identification and cloning of the first mechanosensitive channel, isolated from *E. coli* cell envelope (MscL). The sequence predicts a unique 15-kD protein with highly hydrophobic core and a hydrophilic C-terminus. This project combines structural and functional studies of this first identified mechano-sensitive protein using advanced molecular and biophysical approaches. We will study the topology of protein folding and the stoichiometry of functional channel assembly with biochemical and molecular techniques. Functional roles for certain protein domains will be determined by site-directed mutations followed by functional patch-clamp assays and computer analysis of single-channel recordings. We will try to understand the nature of interactions within the channel conferring a mechanical compliance to the protein complex. Validation of the elemental principles of mechanosensitive protein functioning in eucaryotic MS channels, found in this relatively simple system, will be the final goal of this project.

MscL, a large conductance bacterial mechanosensitive channel, remains the most amenable model for molecular studies of the elemental mechanisms of mechanosensation. During FY96, I achieved substantial progress in structural and functional studies of MscL using predominantly biophysical and biochemical methods. The following aspects of the project have been approached and either partially or entirely solved. I found that MscL is located in the inner membrane of the Gram-negative bacterial cell, which then led to the successful determination of the membrane topology (folding) of the MscL subunit using protein fusion technology. MscL was shown to span the membrane twice with both termini in the cytoplasm. Using a combination of molecular (tandem ORF generation) and biochemical (cross-linking) approaches, we determined that the the active MscL complex is a hexamer. I have begun a thermodynamic description for MscL gating driven by membrane tension by both exprimental and theoretrical approaches. The spatial parameters for the protein conformational transitions and the free energy have been estimated. Determination of topology and the multimeric state of MscL will make possible the detailed computer modeling of this unique molecule, the only way we can envision the molecule in the absence of the crystal structure, and thus predict the functionally-important domains. The results obtained provide a strong framework for experimental validation of structural and theoretical predictions, thus answering the most important question regarding MscL function: what is the nature of intramolecular interactions that set the threshold for force detection by this protein?

This research leads to understanding of very basic mechanisms of force detection by specific biological macromolecules in living organisms. It relates to primary mechanisms of mechanosensation, which encompass a wide range of phenomena from simple osmoregulation in bacteria to complex phenomena such as gravitropism in plants and balance and hearing in humans. MscL represents the first identified and characterized mechanosensory system in bacteria. It provides a highly useful system for a wide range of molecular, biochemical, and biophysical experiments and should be considered as a model. It is difficult to forsee any specific biomedical application of these basic studies within two or three years.

FY96 Publications, Presentations, and Other Accomplishments:

Blount, P., Sukharev, S.I., Moe, P.C., Nagle, S.K., and Kung, C. (review) Towards an understanding of the structural and functional properties of MscL, a mechanosensitive channel in bacteria. Biol. Cell, 87, 1-8 (1996).

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Ermakov, Yu.A., Averbakh, A.Z., Lobyshev, V.I., and Sukharev, S.I. Effects of Gadolinium on electrostatic and thermodynamic properties of lipid membranes. Biophys. J., 70, A96 (1996).

Sukharev, S.I., Blount, P., Martinac, B., and Kung, C. "MscL, a mechanosensitive channel in *Escherichia coli*" in "Organellar Ion Channels and Transporters." Society of General Physiologists, Proceedings of 49th annual symposium. Eds: Clapham, D. and Ehrlich, B. The Rockefeller University Press, pp. 133-141 (1996).

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Gravitational Effects on Signal Transduction

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Project Identification: 199-40-17-08
Initial Funding Date: 3/96
FY 1996 Funding: $129,520

Solicitation: 95-OLMSA-01
Expiration: 2/99
Students Funded Under Research: 1

Task Description:

An understanding of the mechanisms by which individual cells perceive gravity and how these cells transduce and respond to gravitational stimuli is critical for the development of long-term manned space flight experiments. We now propose to use a well-characterized model erythroid cell system and to investigate gravitational perturbations of its erythropoietin (Epo) signaling pathway and gene regulation. Cells will be grown at 1-G and in simulated microgravity in the NASA Rotating Wall Vessel bioreactor (RWV). Cell growth and differentiation, the Epo-receptor, the protein kinase C pathway to the c-myc gene and the protein phosphatase pathway to the c-myb gene will be studied and evaluated as reporters of gravitational stimuli. The results of these experiments will have impact on the problems of 1) gravitational sensing by individual cells, and 2) the anemia of space flight. This ground-based study also will serve as a Space Station Development Study in gravitational effects on intracellular signal transduction.

We examined the association of insulin-like-growth factor-I (IGF-I) expression with atrophy in skeletal muscle. Dr. Vernikos (*BioEssays* 18:1029-1037, 1996) published a paper in which literature citations lead to the hypothesis that IGF-I mRNA would be decreased in muscles of non-weight bearing hindlimbs. Surprisingly, we found that the relative abundance of endogenous IGF I mRNA in the gastrocnemius muscle was unaltered by 14 days of non-weight bearing in the mouse. This complements our previous report that indicates that skeletal muscle IGF-I mRNA expression in fast-twitch muscle was unchanged in the atrophic muscles of old rats. Our interpretation is that whereas increased IGF-I mRNA expression may be involved in skeletal muscle hypertrophy, it does not seem to be causal for non-weight bearing atrophy of skeletal muscle. Concurrent with this research, we performed a study to determine whether over-expression of IGF-I would abate or prevent the non-weight bearing muscle atrophy. Male transgenic and non-transgenic mice from the parent strain (FVB) were divided into four groups (n = 10/group): 1) Transgenic, weight bearing (IGF-I/WB); 2) transgenic, hindlimb non-weight bearing (IGF I/NWB); 3) non-transgenic, weight-bearing (FVB/WB); and 4) non-transgenic, hindlimb non-weight bearing (FVB/NWB). Non-weight bearing groups were hindlimb non-weight bearing for 14 days. Body mass was reduced ($P<0.05$) following non-weight bearing in both transgenic IGF I mice (-9%) and FVB mice (-13%). High level expression of IGF-I mRNA was confirmed in the gastrocnemius and tibialis anterior muscles of the transgenic mice. Nevertheless, the mass of the gastrocnemius and tibialis anterior muscles was reduced ($P<0.05$) in both FVB/NWB and IGF I/NWB groups compared to FVB/WB and IGF-I/WB, respectively, and the percent atrophy in mass of these muscles did not differ between FVB and IGF-I mice. Therefore, a high level local expression of IGF-I mRNA in mouse skeletal muscle does not prevent non-weight

bearing induced atrophy of fast-twitch muscle. Because the transgene was not expressed in the soleus muscle of transgenic mice over expressing IGF-I, we were unable to test the effect of its over expression in slow-twitch muscle.

The results obtained thus far are consistent with the physiology of the anemia of space flight and support our proposed further studies of erythropoietins signal transduction pathways. The anemia of space flight is a complex syndrome characterized in part by a blunted response to erythropoietin resulting in reduced red blood cell production. This problem must be addressed. In addition, on Earth we see a similar blunted response in a variety of disease states, including the anemia found in cancer patients. It is possible that these two diverse conditions share some biochemical/molecular defects and that these defects in intracellular signaling can be modeled in the NASA RWV. Our further studies will dessect the signaling pathways triggered by erythropoietin and will identify those that are affected by microgravity. The results of this experimental approach could lead to new therapies for numerous anemic states, both on Earth and in space.

Growth Factors and Tension-Induced Skeletal Muscle Growth

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Project Identification: 199-40-47-03
Solicitation: 93-OLMSA-07
Initial Funding Date: 4/95
Expiration: 3/98
FY 1996 Funding: $210,358
Students Funded Under Research: 8

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 tension/gravity-related skeletal muscle growth at the cellular and molecular level. The synergistic interaction of defined growth factors and mechanical forces in regulating muscle size will be analyzed in detail. Methods will be developed to grow the organoids in modified bioreactor cartridges of the Shuttle's Space Tissue Loss Module. Finally, the feasibility of "myofiber" gene therapy for the treatment of skeletal muscle wasting will be examined by 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. 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 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 (three to four weeks) of these mammalian organoids. Task one of the project was therefore accomplished during the first year of the project. These mammalian organoids have been utilized for cellular and molecular level studies on tension/gravity regulation of muscle growth. Total cellular protein and myosin heavy chain content are significantly reduced with tension release of the orgonoids. Their long-term survival in the modified bioreactor cartridges of the Shuttle's Space Tissue Loss Module has been accomplished in the project's second year to confirm 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. Finally, implantation of growth hormone

secreting muscle orgonoids into syngeneic animals has been found to be an excellent long term cellular delivery "device" for this anabolic growth factor. Thus, tasks two and three of the project has been completed.

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 has been validated in animal models during the second year of the project.

FY96 Publications, Presentations, and Other Accomplishments:

Shansky, J., Chromiak, J., and Vandenburgh, H.H. (abstract) G protein expression in cultured avian skeletal muscle: Effects of developmental age and mechanical stimulation. Mol. Biol. Cell, 6, 352a (1995).

Vandenburgh, H., Chromiak, J., Shansky, J., LeMaire, J., Perrone, C., Rudio, K., and Twiss, C. (abstract) Space flight induces atrophy of tissue cultured skeletal myofibers. ASGSB Bull., 9, 62 (1995).

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Vandenburgh, H.H. (abstract) Tissue engineering skeletal muscle organs by mechanical forces. ASME Transactions (in press).

Vandenburgh, H.H., Chromiak, J., Shansky, J., and Del Tatto, M. (abstract) Initial International Space Station (ISS) definition studies for examining the effects of long-term space travel on tissue cultured mammalian skeletal myofibers. ASGSB Bulletin (in press).

Vandenburgh, H.H., Del Tatto, M. Shansky, J., LeMaire, J., Chang, A., Payumo, F., Lee, P., Goodyeart, A., and Raven, L. Tissue engineered skeletal muscle organoids for reversible gene therapy. Human Gene Therapy, 7, 2195-2200 (1996).

Vandenburgh, H.H., Del Tatto, M., Shansky, J., LeMaire, J., Chang, A., Payumo, F., Lee, P., Goodyear, A., and Raven, L. (abstract) Tissue engineered skeletal muscle organoids for reversible gene therapy. In Vitro, 32, 53A (1996).

Vandenburgh, H.H., Shansky, J., Chromiak, J., LeMaire, J., Payumo, F., and Del Tatto, M. (abstract) Mechanoregulation of muscle development. J. Cell. Biochem. (in press).

Vandenburgh, H.H., Solerrsi, R., Shansky, J., Adams, J. and Henderson, S. Mechanical stimulation of organogenic cardiomyocyte growth *in vitro*. Am. J. Physiol., 270, C1284-C1292 (1996).

Permeability and Gene Expression in Brain Endothelial Cells Exposed to Shear Stress and Differential Pressure

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Project Identification: 199-40-21-10
Initial Funding Date: 2/95
FY 1996 Funding: $81,000

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 1

Responsible NASA Center: JSC

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.

We have completed the studies designed to quantitate macromolecular permeability changes in BMECs subjected to shear stress. The macromolecular permeability properties of an *in vitro* bovine brain microvessel endothelial cell model exposed to physiological levels of flow-induced shear stress were examined. Monolayers of bovine BMECs were grown on porous polycarbonate memebranes and subjected to wall shear stresses of one or ten dynes/cm^2 for 73 hours in parallel plate flow chambers. Periodically during this extended study, the permeability of the monolayers was quantitated by measuring the transmural flux of fluorescently labeled dextrans. The permeability coefficient was determined for dextrans that ranged in MW from four-2,000 kilodaltons. Brain microvessel endothelial cells initially responded to either one dyne/cm^2 or 10 dynes/cm^2 shear stress with a

dramatically increased macromolecular permeability. The maximum fold increases in permeability were a function of the dextran molecular weight. Long-term application of flow resulted in partial recovery of the permeability barrier function for the BMEC monolayers to macromolecules. A manuscript describing these results was submitted for publication.

Shear stress effects on the gene expression in human umbilical vein endothelial cells has also been examined. Since it has previously been identified that sheer stress can cause arachidonic acid metabolism to increase with the resultant increase in prostacyclin, we have examined the gene expression of the enzyme that causes the rate limiting step of this process. Reverse transcription-polymerase chain reaction, RT-PCR, was used to quantitate the levels of PGHS2 mRNA in the cells at various shear stress levels. Shear stresses of 4, 15, and 26 dyn/cm^2 were applied to the endothelial cells for 0.5 to six hours. Our studies showed that the expression of prostaglandin H synthase, PGHS, was increased in endothelial cells exposed to these shear stresses. Time dependent responses resulted in up to four-fold increases in gene expression of PGHS2.

Our studies will increase our understanding of cephalid fluid redistribution relevant to entry into and recovery from the microgravity environment. The ground-based benefit of these studies will enhance our understanding of the blood-brain barrier (BBB) in hypertension and cerebral trauma. Our studies focus on the physical forces at a cellular and molecular level. Using an *in vitro* model system of the BBB offers advantages of decreased complexity and a more experimentally accessible environment and enables the study of shear and hydrostatic pressure effects, independently and together, at the cellular/molecular level. With this level of understanding, the role of shear stress and hydrostatic pressure mechanisms in fluid redistribution will be clarified. Although the effect of shear stress has been studied with some vascular endothelial cells, this study is the first to examine the effects of shear stress on the specialized endothelial cells that make up the BBB. Brain microvessel endothelial cells differ biochemically from those in other vascular endothelia. Although similarities exist, brain microvessel endothelial cells may be modulated differentially by shear and pressure forces as compared to other endothelial cells. In addition, the effects of hydrostatic pressure on endothelial cells, blood brain barrier-type or other cells, have not been examined in detail. Therefore, the studies described offer unique opportunities to examine the effects of these physiologic forces on gene regulation in the specialized endothelial cells of the BBB. This knowledge about the BBB may be useful in developing treatments for cerebral trauma/edemas as well as for space motion sickness, or understanding the effects of hypertension. Therapeutic approaches may be developed based on a better understanding of the permeability properties of the BBB. Alternative physical or pharmacologic methods may be indicated as a result of this research.

FY96 Publications, Presentations, and Other Accomplishments:

McCormick, S., McIntire, L.V., and Whitson, P.A. Regulation of prostaglandin H synthase gene expression by shear stress. Institute of Biosciences and Bioengineering/Biochemistry and Cell Biology Presentation at Rice University, Houston, TX (April 20, 1996).

Effects of Silver and Other Metals on the Cytoskeleton

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Funding:

Project Identification: 199-40-57-12
Initial Funding Date: 2/95
FY 1996 Funding: $127,717

Solicitation: 93-OLMSA-07
Expiration: 2/98
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.

What has been accomplished thus far?

a. Recently, Tsukube et al. (1995. Tetrahedron Lett. 36: 2257-2260) described "ionophores exhibiting perfect Ag^+ ion selectivity." We obtained samples of three of these compounds (Podands A, B, and C) from Dr. Tsukube and applied them to the fertilized eggs of *Ilyanassa obsoleta* in sea water in the presence and absence of Ag^+ to determine if the cellular sensitivity to Ag^+ was increased by the presence of a Ag^+-selective ionophore (which would be expected to transport the selected ion, in this case Ag^+, across the plasma membrane and into the cytoplasm). Our results indicated: 1) Podands A, B, and C were only sparingly soluble in seawater, even in the presence of relatively high concentrations of DMSO; 2) Podands A, B, and C, alone, in seawater (+/- DMSO) did not appear to affect development significantly compared with seawater alone; and 3) Podands A, B, and C, in the presence of Ag^+, did not affect cellular response to Ag^+, compared with response to Ag^+ alone. Conclusions: Podands A, B, and C may not have been present in high enough concentrations in the plasma membrane to participate in, or alter, cellular responses to Ag^+. Alternatively, Ag^+ may affect cell shape changes by interacting with components on the external surface of the plasma membrane, without having to enter the cell at all. No further work with these podands is planned.

b. Recently, Cramer and Mitchison (1995. J.Cell Biol. 131: 179-189) described the effects of butanedione monoxime (BDM) on cellular shape changes. BDM is a known inhibitor of muscle myosin and non-muscle myosin ATPases. The ATPase site of myosin could conceivably be a site to which Ag^+ might bind and thereby

inhibit myosin-dependent shape changes. We therefore determined if cellular shape changes in fertilized eggs of *Ilyanassa obsoleta* would be affected by BDM in the same concentration range demonstrated by Cramer and Mitchison so as to inhibit cell spreading in (mammalian) PtK2 cells (10 mM) and generate the types of cell shapes characteristic of treatment with Ag^+. Result: at concentrations of 10, 15, and 20 mM, BDM elicited abnormal cell shapes that greatly resembled those seen in response to Ag^+. Conclusion: Ag^+ may inhibit cell shape change by inhibiting myosin ATPase used for changing cell shape.

c. Although we know most about the effects of Ag^+ on the cellular shape changes and on the cytoskeleton of fertilized *Ilyanassa obsoleta* eggs, we recognize that the significance of those results lies in the implication that Ag^+ might have similar effects on human cells. We reasoned that Ag^+ in drinking water of astronauts would interact first with the epithelial cells lining the human gastrointestinal tract. Therefore during the past year, we tested the effects of Ag^+ on established lines of cells *in vitro* to determine its effects on cell proliferation and on the microtubules of the cytoskeleton. Results: 1) The normal embryonic human intestinal epithelial cell line, FHs74int, divides more slowly in 1 μM Ag^+; stops dividing in 5 μM after 3 days, in 7.5 μM Ag^+ after 5-7 days and in 10 μM after 12-24 hrs. Operationally, however, these cells grew too slowly to allow convenient experimentation. 2) The transformed human intestinal epithelial cell line, CaCo 2BBe, grows more rapidly than FH74int and shows inhibition of proliferation at 5 μM Ag^+. When the array of microtubules is examined in CaCo 2BBe cells, results indicate no difference in the rate of depolymerization of microtubules in the presence of Ag^+, no difference in repolymerization rate, and no difference in the density or pattern of the equilibrium array of microtubules in such cells in the presence of Ag^+. 3) The effects of Ag^+ on cell division also were examined in an immortalized line of quail ventricular cardiomyocytes, TD5, which showed inhibition of proliferation at 10 μM Ag^+. Conclusions: Proliferation of mammalian and bird cell lines is inhibited in the presence of 1-10 μM Ag^+. In addition, we currently have no data from vertebrate cells indicating any obvious effect of Ag^+ on the structural stability or dynamics of microtubules. However, we plan one further test of our hypothesis that Ag^+ affects microtubules.

What questions have been answered?

1. The Ag^+-specific ionophores of Tsukube et al. (1995) neither enhanced nor suppressed the effects of Ag^+ on the cellular shape changes of fertilized *Ilyanassa* eggs.

2. An agent known to inhibit myosin ATPases inhibits the cell shape changes of fertilized *Ilyanassa* eggs at the same concentrations.

3. A variety of mammalian and avian established cell lines display inhibition of proliferation at approximately the same concentrations of Ag^+, but so far the one cell line examined for microtubules has failed to reveal any effect of Ag^+ on the microtubule arrays of those cells.

4. Fertilized eggs of *Ilyanassa obsoleta* contain mRNAs for at least one type of myosin. The nucleotide sequence determined was used to translate peptides specific for *Ilyanassa* myosin. Those peptides were injected into chickens and these animals have layed eggs whose yolks contain antibodies that react not only with the peptide antigens, but also with proteins from the *Ilyanassa* cells. Such antibodies promise to be useful for recovering myosin from these cells to allow determination of the effect of Ag^+ on the ATPase activity.

What new questions have arisen?

Although our initial experiments on vertebrate cells detected no obvious effect of Ag^+ on microtubules, it will be important to examine possible effects of Ag^+ on the microtubules of a few other vertebrate cell types, such as embryonic nerves and cardiac muscle cells.

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

1. Using fertilized *Ilyanassa obsoleta* eggs, we will examine possible effects of Ag^+ on myosin ATPases.

2. Using vertebrate cells, we will continue to examine the effects of Ag^+ on cell proliferation and on the microtubules and microfilaments of the cytoskeleton. The two cell-types that we will study first will be: a) the sensory nerves that grow from dorsal root ganglia of embryonic chicks *in vitro*, and b) cardiomyocytes from the ventricles of embryonic chickens. In both cases, these specialized cell-types have elaborate cytoskeletons that require both microtubules and microfilaments (the latter by interacting with myosin) to accomplish neurite outgrowth in the case of dorsal root ganglia sensory nerves, and to accomplish assembly and regular contraction of the cardiac muscle sarcomeres in the case of the ventricular myocytes.

Plans for Year 3

In addition to those anticipated experiments outlined above, we will continue to try alternate techniques for immobilizing fertilized *Ilyanassa obsoleta* eggs without killing them. Such immobilization of viable cells is required for testing Hypotheses #2, #3, and #4 of the original proposal.

Silver metal (Ag^0) 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 microorganisms or infections in your body" and to "gradually build your immune system." However, physicians have warned recently of the long-term danger of consuming Ag-containing solutions for long periods of time (can cause a generalized deposition of Ag^0 in the skin and mucous membranes which remains permanently as grey depositions (Ag^0 metal), a condition known as argyria). Moreover, many organ systems and specific enzymes are inhibited by Ag^+ at concentrations equivalent to those being used in the applications above. Our research represents a focused attempt to understand the molecular mechanism(s) involved in the toxic effects of Ag^+ on animal cells.

FY96 Publications, Presentations, and Other Accomplishments:

Conrad, A.H. and Conrad, G.W. (abstract) Molecular characterization of myosin and the sodium-proton antiporter in *Ilyanassa obsoleta*. Bulletin Mt. Desert Island Biol. Lab., 35, 17-18 (1996).

Conrad, A.H., Koo, S.J., Hebert, G.L., and Conrad, G.W. (abstract) Expression and localization of myosin in fertilized eggs and adult tissues of *Ilyanassa obsoleta*. Bulletin Mt. Desert Island Biol. Lab., 36 (in press).

Conrad, G.W., Janasek, M.J., Martinez, N.M., and Conrad, A.H. (abstract) Mechanisms of silver ion (Ag+) toxicity in fertilized eggs of *Ilyanassa obsoleta*. Bulletin Mt. Desert Island Biol. Lab., 35, 5-6 (1996).

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 1996 Funding: $ 140,337
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. Through this project, we plan to determine whether the distribution of cytoplasmic morphogenetic determinants, and thus the developmental fate of blastomeres, is altered by novel gravitational fields by either tilting them or rotating them in a horizontal clinostat. We then plan to compare the control and experimental embryos with respect to blastomere fate (by lineage tracing with fluorescent dextrans), blastomere commitment and autonomous differentiation potential (by transplantation and culture), and distribution of cytoplasmic morphogens (by *in situ* hybridization). These three approaches, when applied in tandem, will provide a definitive test of the hypothesis that the distribution of cytoplasmic morphogenetic determinants and thus the developmental fate of blastomeres can be altered by novel gravitational fields.

In the 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 which are subjected to 90° rotation before the first cleavage. We found that at the 8-cell stage, the blastomeres changed their normal fate—blastomeres on the top of the embryo 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 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 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.

The cell fate study of the blastomeres in the 90° rotated embryos which started in the first year is completed. A manuscript of the cell fate change at 8-cell stage has been sent to *Developmental Biology* in December, 1996. The indication of the results is that since the cell fate of cleavage stage blastomeres may be determined by their cytoplasmic determinants, the differential cell fate change in the rotated embryos suggests that the cytoplasmic determinants may undergo complicated redistribution under the influence of novel gravitational fields. The paper about the cell fate change of the blastomeres at 32-cell stage is in preparation.

In FY96 we started the experiment II as originally proposed in the grant. That is to determine if the cell fate change in the rotated embryos is cell autonomous or cellular interaction dependant, using cell transplantation and cell culture techniques. The cell transplantation part of the experiments (IIb) is nearly finished. The results demonstrate that blastomeres change their inducing and responding abilities in rotated embryos. Preliminary results from the cell culture experiments (IIa) also showed that blastomeres may change morphological pattern formed in culture after rotation of the eggs during first cleavage. Both experiments indicate that changes in the rotated embryos may be caused by the reorganization of the cytoplasmic components, particularly the relocation of the dorsal determinants, under the effect of gravity.

In FY96, we also started the experiments with horizontal clinostat which simulates microgravity conditions. The fate change from this experimental procedure is not as obvious as from the rotation procedure, as I pointed in the FY95 Task Progress. However, the morphological alterations have already been shown from horizontal clinostat treatment and the investigation into the subtle fate change under microgravity conditions may help to elucidate the mechanism of the morphological alterations under microgravity, such as relocation of the maternally derived dorsal determinants. This part of work will be the major task in the second year of the grant period.

It is indicated by the work thus far that to understand the effect of gravity on the animal development it is important to study at both cellular and molecular levels the reorganization of the dorsal determinants in the early embryos that are subjected to the novel gravitational conditions such as body axis rotation or horizontal clinostat treatment. This will be the focus of our study for the third year of the grant period and hopefully for the years to come.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Huang, S. and Wang, P.H.Z. Cell fate change in axis-tilted *Xenopus* embryo is the result of the cytoplasmic reorganization under the effect of gravity. ASGSB Bull, 10, 36 (1996).

Moody, S.A., Bauer, D.V., Hainski, A.M., and Huang, S. Determination of *Xenopus* cell lineage by maternal factors and cell interactions. Current Topics in Development, 32, 103-138 (1996).

The Effects of Gravitational Loading and Vibration on Vestibular Ontogeny

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Funding:

Project Identification: 199-40-17-01
Solicitation:

Initial Funding Date: 10/92
Expiration: 9/96

FY 1996 Funding: $0
Students Funded Under Research: 8

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

The specific aim of the research is to test the working hypothesis that gravitational loading and/or seismic vibration do 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 2-G 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 (2-G) or whole body vibration during ontogeny significantly change vestibular compound action potential thresholds and/ or activation latencies in the chicken?

Task progress for FY96 includes:

1. The adequate stimulus for vestibular responses is linear jerk (time drivative 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 stimuli for gravity sensors. The data form the basis of a manuscript entitled "Linear jerk as the adequate stimulus for short latency vestibular responses" submitted to the *Journal of Vestibular Research.*

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" (In press: *Journal of Vestibular Research).* 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. A cursory report has been published (Jones, T.A. and S.M. Jones. Ontogeny of vestibular compound action potentials in the chicken. Abstracts Society for Neuroscience: Abs#365.12, V21(Part 2), p919, 1995. A complete report is in preparation.

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)). These data form the basis for a manuscript entitled: "Vestibular responses to pulsed linear acceleration in the quail" submitted to the Journal of Comparative Neurology.

6. Gravitational loading and vestibular ontogeny: A complete study was organized to compare vestibular function in animals incubated and hatched at 1-G (normal), 1.2-G (rotation control) and 2-G. This study was initiated and the data are now being analyzed and a manuscript is in preparation.

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-G environment? Gravity is markedly decreased during space flight and the vestibular system of developing embryos subjected to the microgravity environment might develop abnormally (Jones 1992; Jones et al., 1991, 1993; Fermin et al., 1996). Gravitational field strength can also be increased using a centrifuge. Studies evaluated 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.

FY96 Publications, Presentations, and Other Accomplishments:

Estrem, S., Bechtel, J., and Jones, T.A. (abstract) Vestibular evoked responses in the evaluation of ototoxicity in the chick. Assoc. for Res. in Otolaryngology, Abs #83, (1996).

Fermin, C.D., Martin, D.S., Jones, T.A., Vellinger, J., Deuser, M., Hester, P., and Hullinger, R. Microgravity in the STS-29 Space Shuttle Discovery affected the vestibular system of chick embryos. Histol. Histopath., 11 (2), 407-426 (1996).

Jones, S.M. and Jones, T.A. Short latency vestibular evoked potentials in the chicken embryo. J. Vestibular Res., 6(2), 71-83 (1996).

Jones, S.M. and Jones, T.A. (abstract) Functional and pharmacological attributes of the avian cochlear microphonic and compound action potential. Assoc. for Res. in Otolaryngology, Abs #299 (1996).

Pinheiro, A.D., Stanley, C.M., David, H., Maruniak, J.A., Estrem, S., and Jones, T.A. (abstract) C-fos expression following acoustic stimulation in chicks, *Gallus domesticus*. Assoc. for Res. in Otolaryngology, Abs #357, p90, (1996).

Pinheiro, A.D., Stanley, C.M., David, H., Maruniak, J.A., Estrem, S., and Jones, T.A. (abstract) Linear head acceleration induces c-fos expression in the brainstem of the chick, *Gallus domesticus*. Assoc. for Res. in Otolaryngology, ABS #701, p176, (1996).

Altered Gravity and Early Heart Development in Culture

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Funding:

Project Identification: 199-40-27-24
Solicitation: 95-OLMSA-01
Initial Funding Date: 1/96
Expiration: 12/96
FY 1996 Funding: $85,436
Students Funded Under Research: 2

Task Description:

The effect of altered gravitational forces on the early development of the heart in the chick embryo will be examined using pre-cardiac explant tissues incubated in culture. The extracellular matrix glycoprotein fibronectin is known to be important in the heart development process. The goal of the proposed work is to obtain a new understanding of the effect of altered gravity on the production and deployment of fibronectin during the early development of the heart, and to determine whether or not these alterations lead to impaired cardiac myogenesis or morphogenesis. Previous experiments have shown that the microgravity of space flight delays the accumulation of contractile myofibrils in these tissues during an early sensitive period, and preliminary data suggest that coincident with this fibronectin may be greatly restricted.

Immunostaining, immunoassay, and electron and light microscopy will be used to achieve the following specific objectives: to determine, for precardiac explants developing under conditions of microgravity, unit gravity, and hypergravity: a) the overall tissue morphology, the immunolocalization of fibronectin, b) the measured accumulation and production of fibronectin, c) the ultrastructural characteristics, and d) the ability to develop spontaneous contractions. The proposed work aims to elucidate the mechanism by which gravity affects cells during their development in an experimentally accessible, well-characterized system, and it is relevant to NASA's mission in understanding the effects of gravity on animal development processes.

More than 200 precardiac explant pairs have been dissected and cultured as controls and bioreactor rotated (simulated microgravity) experiments in pairs. Each has been assessed for general morphology, and the ability to contract spontaneously. Many have been immunostained with antibodies to fibronectin and several images of these sections have been captured for computer-assisted image analysis. Electron microscopy has been carried out on several pairs of explants. All of these procedures are continuing as we conduct approximately weekly experiments.

All precardiac explants from the initial experiment were photographed using phase contrast microscopy. These and all others from subsequent experiments have been carefully observed. They possess the expected size and shape, and a vesicle is present. There have been no observable differences between control and bioreactor rotated explants

Each explant has been observed for spontaneous contractions immediately following the 18 hour culture period. There is a clear inhibition of the development of contractile activity as a result of bioreactor rotation.

Immunostaining of sections of the recovered explants has shown substantial differences between control and bioreactor rotated explants. Although positive staining is present in all, most of the rotated explants showed less intense staining in all areas. To date, 87% of these are less stained than controls.

The control explants showed areas of intense, linear, extracellular staining along epithelia. These are suggestive of basement membrane staining, but the areas were shorter and more discontinuous. Control sections also showed areas of intercellular staining in both mesenchymal tissue and myocardium. Areas of staining with this pattern were seen in sections from rotated explants as well, but they were more restricted. Usually confined to quite small areas of only a few cells, and they were less intense. We have our image analysis system assembled and have worked out the procedures for measuring intensity of red staining with background correction, and for measuring areas of red staining in square microns, both using Adobe Photoshop and NIH Image software. The analysis is proceeding.

Initial immunoassay results from two experiments have shown a possible correlation of tissue fibronectin accumulation with the immunostaining results. Five control and five bioreactor rotated explants were pooled, homogenizer, diluted twofold to create a series of samples, and blotted onto nitrocellulose sheets. Fibronectin purified from adult chicken tissue by immunoaffinity column chromatography was diluted and applied as a standard. Immunostaining and quantitation of these by image analysis (NIH Image) have produced stained slots with a linear range of assay response. In these experiments we have observed an average 24% reduction in fibronectin. This would suggest a reduction of approximately 28 picograms of fibronectin per explant during bioreactor rotation. We are working to standardize the measured quantities of fibronectin per unit of DNA, in order to check whether the differences we have observed are authentic on a per cell basis.

Seven control and nine bioreactor rotated explants have so far been analyzed by electron microscopy under the supervision of Dr. Spooner at Kansas State University. More will be analyzed to achieve reliable results. An improvement in fixation has been achieved with addition of 2% tannic acid to the glutaraldehyde/formaldehyde fixative, and change to cacodylate buffer. The cells that have been viewed exhibit the expected ultrastructure. Myofibrillar structures are apparent and are characteristic of the expected degree of development (Hamburger-Hamilton stage 6 or 7 plus 18 hours yields approximately stage 11). No differences between control and rotated explants have been apparent at this time.

Therefore, at this time, we have answered three questions. First, it is clear that rotation in the HARV bioreactor simulates microgravity effectively and we feel confident the instrument provides a good model system. Second, it is apparent that rotation is delaying or inhibiting the development of spontaneous contractions in the tissues. Third, the amount and distribution of fibronectin is diminished from microgravity exposure. Our continuing experiments and particularly our image analysis measurements and electron microscopy will refine these conclusions further.

Our results may raise a new question concerning the production and assembly of myofibrils. Van Twest et al (1995) found delay or inhibition of myfofibrils in space flight explants taken from stage seven and especially stage six explants, yet so far, we have not found evidence for this effect in bioreactor experiments. Thus we now ask, does the microgravity environment of space shuttle flight differ from that of the bioreactor with regard to this dimension of myogenesis? Completion of our experiments may yield additional data that will help answer this question.

We have also encountered the possibility that certain vibrations may prevent the inhibitory effects of bioreactor rotation. The bioreactor we have been using was found to rotate unreliably at first because of its electric motor. Replacement of the original motor at Synthecon, Inc. (Houston, TX) solved the problem but resulted in considerably louder rotation. Subsequent experiments showed no differences between control and rotated explants in the development of contraction ability. The instrument was returned to Synthecon and dampened, and now the inhibition of development of contractions is apparent again. Future work may allow the investigation of the possible effect of vibrations on the cell surface mechanotransduction apparatus that is apparently sensitive to vibration as well as gravitation.

In the immediate future, we plan to determine fibronectin concentration in explant tissue more definitively, and also in the culture medium. If the tissues do have less fibronectin, we wish to know if it is being secreted into the medium in greater amounts, or if total production is less. Our results thus far with microgravity exposure also suggest that hypergravity exposure of precardiac explants will be important to investigate—if the gravity receptors are sensitive to microgravity, then interesting results might be seen in hypergravity. Experiments using centrifugation at the NASA-Ames Research Center are being planned.

All life has evolved at unit gravity. This has surely shaped biological systems, especially fundamental cellular activities and developmental processes. But it has been difficult to know how—to know what components of living matter are the targets for perception and adjustment to gravity, particularly at the cellular level. This is because we cannot easily manipulate gravity. However a viable hypothesis has recently begun to accrue experimental support: that the complex network of macromolecules comprising the cellular cytoskeleton and the extracellular matrix, connected to one another through the cell surface via receptors and other membrane components, and serving to mediate information transfer and dynamic adjustment for the cell, is sensitive to gravitational force (Spooner, 1994).

Fibronectin is an abundant extracellular matrix molecule that links the cell surface to the matrix. It is produced by cells and deposited at their surfaces where adhesions, migrations, and signalling to other cells will take place, events crucial to proper development of embryos. Formation of the embryonic heart is an experimentally accessible event where such adhesions, cell migrations, and signaling will occur. Preliminary results suggest a sensitivity of heart myogenesis and fibronectin production to gravity. If the sensitivity to gravity and the importance in heart development of fibronectin can be determined, we will derive a more complete understanding of how gravity influences normal animal development and ability to reproduce.

FY96 Publications, Presentations, and Other Accomplishments:

Lwigale, P., Cenning, J., and Wiens, D. (poster) Altered gravity and early heart development in culture. American Society for Gravitational and Space Biology Annual Meeting, Charlotte, NC (October, 1996).

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 1996 Funding: $0
Students Funded Under Research: 3

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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*.

[1]. Studies of heat shock or stress proteins (hsp) expression in mice exposed to heat shock and hypoxia as ground-based models of stress.

The long-range goal of our experiments is to elaborate sensitive markers and tests for cell injury in response to potential hazards of actual space flight. One of the promising candidates for sensing subtle changes in cellular homeostasis after exogenous stress are hsp. Since opportunities for flight experiments are severely limited, we have performed ground based studies for establishing the possible use of hsp as sensitive molecular markers of cellular abnormalities after exposure to potential hazards of space flight.

We have now begun to characterize the spatio-temporal pattern of expression of several different hsp family members in the mammalian brain using two models of exogenous stress: acute exposure to heat shock and oxygen depletion. After specified periods of time animals were euthanized with CO_2 and tissues were processed for immunohistochemistry.

The experiments showed that both heat shock and hypoxia treatment elicited heat shock responses in the mammalian brain. The strongest induction was observed at eight and 16 hours after stress. Expression of Hsp70-inducible species was detected in neuronal populations of cortex and hippocampus. Microvessels and glial cells expressed Hsp70s throughout the brain. Hsp32, encoding heme-oxygenaseI, which catalyzes conversion of heme to biliverdin, was expressed after both hypoxia and heat shock in neuronal populations of

cortex, hippocampus, cerebellar cortex, and reticular formation of brainstem. The most interesting data were obtained with antibodies specific for a small stress protein, Hsp25. The expression of this protein was restricted to trigeminal and facial motor nuclei, which innervated skeletal muscles of face and jaws. No other neurons in the brain expressed this protein, which was present in control and was highly induced in tissues after heat shock. Moreover, the immunoreactivity for Hsp25 was observed also in axons and fibers of cranial nerves V and VII respectively, indicating the possible involvement of Hsp25 in fast axonal transport. The high level of expression of Hsp25 in facial and trigeminal motor nuclei indicates its important role in protecting these regions from exogenous stress.

Thus, our experiments suggest that hsps can serve as a sensitive markers of cell injury in different cell populations and in particular, in different regions of mammalian brain after exogenous stress. Moreover, Hsp25 can be used as a unique and specific marker for cell injury in facial-trigeminal neuronal system. We are now extending this analysis to determine if these reagents are useful for detecting Hsp25 in a non-mammalian species, the *medaka*. We will also extend this analysis to examining embryonic stages of mouse development, to ascertain if there are differences in response to external stress at different stages of embryogenesis.

[2]. Studies on the pattern formation homeobox gene *Hoxa-4* in the fish *medaka*.

a. Cloning and characterization of the *medaka Hoxa-4* homologue: A 350 bp Hinc II-Xba I fragment of mouse *Hoxa-4*, including part of the 3' intron, the second exon and the homeobox sequences, was used to screen at reduced stringency a *medaka* fish genomic library. Positive clones were subjected to a final round of plaque purification at high stringency. The screening of genomic *medaka* fish library yielded a positive 13 kb Not I fragment which was then cloned into pBluescript SK(+). Sequencing of a 2kb region containing the whole coding sequence of *medaka Hoxa*-4 revealed the existence of 59.5% and 68% conservation with mouse *Hoxa-4* on the nucleotide and aminoacid level respectively. The degree of conservation reached 79.3% in the homeobox. Additional sequences conserved between *medaka*, mouse, and chicken, were also found in the 5' non-coding region, including two potential homeodomain binding sites.

b. Characterization of the pattern of expression of *medaka Hoxa-4*: A 366 bp Sac I-NcoI fragment, containing most of the first exon coding sequence, was selected and cloned into pBluescript SK(+), in order to generate ^{32}P-labeled riboprobe for use in *in situ* hybridization analysis. RNA was collected from *medaka* embryos at various stages of embryogenesis. Nothern blot analysis of total RNA fractions from staged medaka embryos revealed the existence of three transcripts of 1.6, 2.4, and 6.5 kb. These transcripts were undectable at stage 16, corresponding to the late gastrula stage just preceeding the neurula stage. All three were present at low level at stage 21, when embryos possess 6 somites and the brain and octic vesicle formation initiates. The 6.5 kb transcript expression reached a peak around stage 25 and was strongly reduced at stage 33. The 1.6 kb and, at a much lower level of expression, the 2.4 kb transcripts, showed a stable signal intensity from stage 25 to 33, when the embryo is almost ready to hatch.

This research seeks to understand the effect of space flight and microgravity on vertebrate development, in particular on the development and function of the central nervous system (CNS). The overall goal of the proposed study is to identify and evaluate sensitive molecular and cellular markers of vertebrate morphogenesis in order to assess the effects of the altered environment of space flight on embryonic and post-embryonic development. The hypothesis to be examined is that embryonic development (and neural development in particular) will be affected, potentially in a subtle but biologically significant manner, by exposure of the animals to the environment of space, and further, that this response will be different at different stages of embryonic and post-natal development of the animal. While our research does not seek to develop directly new therapeutics or protocols of alleviating symptoms of a disease or malady on Earth, it is extremely relevant to understanding the effects of altered environments on normal and abnormal human and animal development and in the etiology of pathological conditions that can occur, in particular, under stress. That is, this research will yield new understanding of basic biological processes, such as the regulation of gene expression in response to exogenous stress during early development and the molecular mechanisms involved in adaptation to microgravity. The success of developmental processes including fertilization, embryonic development and

maturation determines the ability of a species to survive in a certain environment. Space flight environment includes several hazards that potentially are able to affect developmental processes such as radiation, alterations in atmospheric pressure, prolonged toxic exposure, and microgravity. The impact of this research 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.

FY96 Publications, Presentations, and Other Accomplishments:

Murashov, A.K. and Wolgemuth, D.J. Differential localization of the sense and antisense *hsp*70.2 gene transcripts in neuronal populations of distrinct regions of the brain. Mol. Brain Res., 37, 85-95 (1996).

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Space Biology Research Project

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Funding:

Project Identification: 199-99-17-03
Initial Funding Date: 12/94
FY 1996 Funding: $0

Solicitation:
Expiration: 11/00
Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 this requirement, and others are in progress.

FY96 Publications, Presentations, and Other Accomplishments:

Behringer, F. Altered auxin redistribution the *lazy*-2 gravitropic mutant of tomato. Gordon Research Conference on Gravitational Effects on Living Systems, Colby-Sawyer College, New London, NH (July 14-19, 1996).

Behringer, F. (poster) Genetic analysis of the *lazy*-2 mutant. Molecular Biology of the Tomato Conference, University of California, Berkeley (August 1-4, 1996).

Correia, M.J., Ricci, A.J., and Rennie, K.J. Filtering properties of vestibular hair cells: An update. Annals of the New York Acad. Sci., 781, 138-149 (1996).

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Ricci, A.J., Rennie, K.J., and Correia, M.J. (abstract) An electrophysiologic comparison of type I avian hair cells of different inner ear end organs. Eighteenth ARO Midwinter Meeting, 18:162 (1995).

Plasmadesmata and the Control of Gravitropism

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Project Identification: 199-40-57-22
Initial Funding Date: 10/94
FY 1996 Funding: $

Solicitation: 12-10&11-92/GB
Expiration: 12/95
Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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.

During the past year, research was pursued along two major lines. The first was to develop a system for measuring the pH of the apoplast using confocal microscopy and pH-sensitive dyes. The importance of this for the studies on the control of gravitropism is that there is clear evidence that the apoplastic pH is a major determinant of the amount and direction of polar auxin transport. In gravitropism the normal longitudinal polar auxin transport system is redirected so that it moves laterally to some extent. It is known that one component of the polar auxin transport is the ability of cells to take up auxin from the apoplast, and that is dependent on the pH of the apoplast. If a pH-gradient were set up in the apoplast across a horizontal organ, it would facilitate the lateral transport of auxin. The problem is that there has not been a way to measure the apoplastic pH of different walls across a tissue. We have taken advantage of the fact that the pH-sensitive dyes, D-NERF and Cl-NERF, change their fluorescence in the pH range that is expected to occur in the apoplast; namely, 3.0 to 5.5. In order to quantitate the pH, it is necessary to be able to ratio the fluorescence of the NERFs to some other pH-insensitive dye. We are using Texas Red as the second dye. The idea is to infiltrate the two dyes into the apoplast of plant tissues, and then use the ratio of fluorescence of the two to measure the apoplastic pH. We are concentrating on the *Avena* coleoptile, since it is an organ that shows strong gravitropism and has a simple anatomy. The immediate goal is to add an agent that will penetrate into the tissues, and see how long it takes to change the pH in the apoplast as one progresses into the tissue. The agent being used is fusicoccin, since it causes rapid acidification of the apoplast. To date, much of the effort has been directed towards validating the system and overcoming technical problems that have arisen. But the results are encouraging.

The second project concerns the effect of gravity on cell-to-cell communication in the *Avena* coleoptile. Two procedures have been used here. The first is to introduce carboxyfluorescein diacetate, which is non-fluorescent but readily taken up into cells. There is hydrolyzed by esterases to liberate the fluorescent carboxyfluorescein (CF). If one cell becomes fluorescent while its neighbors do not, it indicates that the plasmodesmata connecting the cells has not been open to CF. The second procedure is to inject a fluorescent dye directly into a cell and observe whether or not it moves to neighboring cells. In the *Avena* coleoptile, the evidence now indicates that the epidermal cells are effectively isolated from all neighboring cells, and that this isolation is unaffected by gravity. On the other hand, there is a ring of cells underneath the epidermis, subepidermal cells, which are interconnected in the longitudinal direction. There may also be some connection between these cells and the cortical cells inside of them. The small cortical cells surrounding the vascular bundles are symplastically connected to the phloem. Here, gravity seems to play a role as there is increased movement of dye from the phloem into these cells when the tissue is horizontal. In general, these results indicate that cells in an elongating organ, capable of gravitropic curvature, are mostly isolated from each other symplastically. This means that a gravity-induced redirection of the polar auxin transport stream is more likely to be due to a change in location of active auxin efflux carriers.

The Earth benefits of this research fall into two areas: benefits to an understanding of basic biological processes, and benefits to agriculture. Plants consist of a multitude of cells, fixed in position by their walls, and interconnected by plasmodesmata into a "symplast." The plasmodesmata are believed to permit small molecules (ones smaller than about 800 Da) to pass freely from cell to cell. This would include sugars, amino acids, ions, and of course plant hormones. This raises some important questions. How can cells end up differentiating into different cell types when they are contiguous and if they are subjected to the same chemical environment? How could gradients of morphogenetic factors exist if the cells are really freely interconnected? Is there any control of the movement of small molecules through the plasmodesmata? The research being conducted under this task is some of the first work on the cell-to-conductance in growing and developing tissues. Until now, most research on plasmodesmata has focused on one of two systems—mature leaf mesophyll cells, and phloem companion and parenchyma cells. Neither of these is a tissue in which morphogenetic gradients is

expected to play an important role. As a result, our knowledge about the plasmodesmal conductance of developing tissues is limited. The research conducted here indicates that the conductance is far more limited than had been realized. It indicates that developing cells may exert real control over the ability of hormones to move from cell to cell. It is the start of what should prove to be an important area of plant research.

Plasmodesmal conductance is an important topic in agriculture for several reasons. First, the spread of viruses in plants from cell to cell occurs through the plasmodesmata. Each virus codes for a movement protein which causes a huge increase in the size exclusion limit of the plasmodesmata and carries the viral nucleic acid through the plasmodesmata. But how do these movement proteins exert their effect? Until we know far more about the control of the plasmodesmal conductance we cannot answer that, or devise effective ways of preventing the viral nucleic acid-movement protein from actually moving. A second important question is how the growing meristems of root and shoot are supplied with the nutrients needed for growth. It has been postulated that in the root, sugars unloaded via plasmodesmata from the sieve tubes into parenchyma cells then move to the meristem via the plasmodesmata. But if the plasmodesmata are really closed, as my research suggests, alternative movement pathways most occur. The results of my work may point the way to future research which will provide answers to these questions.

Plant Gravitropisms and the Role of Expansins

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Funding:

Project Identification: 199-40-57-44
Solicitation: 95-OLMSA-01
Initial Funding Date: 11/95
Expiration: 10/96
FY 1996 Funding: $ 122,940
Students Funded Under Research: 2

Task Description:

The general goal of this research is to elucidate the cellular and molecular mechanisms by which gravity alters the growth of plants. Plant gravitropisms involve an asymmetry in the rate of cell enlargement and wall expansion on the two sides of the bending organ. However, relatively little is known about the detailed molecular mechanisms by which asymmetric cell wall expansion is established and controlled during gravitropism. Published work indicates that an asymmetry in "acid growth" may be partly responsible for the growth changes. The experiments proposed here build on our recent discovery of the proteins ("expansins") that mediate this acid-growth mechanism. We propose experiments to analyze expansin gene expression, expansin protein activity, and wall sensitivity to expansin action during gravitropism of cucumber and *Arabidopsis* seedlings. We will use expansin mutants and transgenic plants with altered expansin levels to characterize the sensitivity of gravitropic bending to expansin content. Furthermore, we will use PCR to clone and sequence expansins from divergent taxa, as a means of discovering conserved (functional) domains of the protein and as a prelude to future work with these species. The results should advance our understanding of the molecular machinery that controls plant cell expansion in general and how gravity (via the gravitropism transduction pathway) interacts with this machinery to modulate plant cell expansion.

Our principal objectives for this year included:
1. Northern analysis of cucumber hypocotyls to analyze expression of expansin genes;
2. Analysis of expansin protein activity in cucumber hypocotyls during gravitropism; and
3. Analysis of wall susceptibility in cucumber hypocotyls during gravitropism.

Below are described in brief our principal efforts and results:

1. Northern analysis of expansin mRNA in cucumber hypocotyls;
a. The patterns of expression (mRNA abundance) along the length of the cucumber hypocotyl were measured for CuExS1 and CuExS2. Highest expression for both genes was found in the region of maximal growth, with reduced expression in more basal regions. The pattern of mRNA expression and growth along the hypocotyl were found to be nearly identical.
b. Initial experiments with auxin induction of growth indicated that the two expansin genes are differentially sensitive to auxin action. One gene is rapidly up-regulated, whereas the other is hardly affected with 3 h. Since auxin asymmetry is generally implicated in gravitropic responses, these results were of great interest.
c. Initial experiments with bisected hypocotyls indicated that RNA abundance and quality were potential

problems; we have modified our protocols for tissue harvesting and further experiments are planned for this fall to overcome these initial obstacles. Because auxin induces one of the expansin genes (d, above), we are eager to carry out this experiment, which will shed light on the role of auxin-induced expansins in the growth response during gravitropism.

2. Tissue print analysis of expansin mRNAs in cucumber hypocotyls
We tried to use tissue printing to detect the pattern of expansin expression in the hypocotyl during gravitropism. This method involves bisecting the hypocotyl during various stages of gravitropic bending and pressing the cut surface onto specially-treated nitrocellulose membranes to transfer the RNA to the membrane, which is subsequently hybridized with 32P-labelled expansin probes and then visualized by autoradiography. We obtained signals, but they were relatively weak and the patterns were not convincing enough for us to draw firm conclusions. Therefore, the Northern analysis (described above) will be used, at least until a better method of tissue printing is developed.

3. Analysis of expansin activity in bisected hypocotyls during gravitropism
For these experiments, we placed etiolated cucumber seedlings in a horizontal position for 0-90 min, and at intervals, the hypocotyls were harvested, bisected, frozen, and analyzed for acid-induced wall extension - a hallmark of expansin action. No differences in extension response were observed between top and bottom halves of the hypocotyl.

The simplest interpretation of these results is that expansin activity/abundance is not altered in a stable way during cucumber hypocotyl gravitropism. Another possible interpretation is that the assay lacks adequate sensitivity to detect significant but subtle changes. For example, the change in expansin activity might not survive the procedures used for tissue harvesting and assay (bisection, freezing, thawing, clamping in buffered solution, etc.). We are currently testing out alternative procedures to circumvent some of these potential problems (e.g. rapid freezing in -20 glycerol).

FY96 Publications, Presentations, and Other Accomplishments:

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Keller, E. and Cosgrove, D.J. Expansins in growing tomato leaves. Plant J., 8, 795-802 (1995).

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Cellular Specificity in Arabidopsis *Root Gravitropism*

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Project Identification: 199-40-57-27
Solicitation: 91-OSSA-15
Initial Funding Date: 2/95
Expiration: 2/98
FY 1996 Funding: $93,000
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 is to use existing technology to compare the location of the motor cells in wild type and starch-deficient mutants of *Arabidopsis* and tobacco, to construct the new closed loop feedback system for control of seedling orientation, and to complete software required for data analysis. We would also plan to begin the comparison of zones of gravisensing in wild type and starchless mutants of *Arabidopsis* and tobacco during the first year. During the second year we would complete the study of localization of zones of gravisensing and begin a study of the role of the extracellular matrix (as an alternative to the amyloplast sedimentation hypothesis) in gravisensing. The emphasis during the third year would be on the analysis of gravitropism in auxin overproducer transgenics and in auxin/gravitropism response mutants of *Arabidopsis*.

During the past year we identified subpopulations of cells in the DEZ of roots of both the WS wild type of *Arabidopsis* as well as the *rgr1* mutant derived from this wild type. We have determined that the auxin responsiveness is dependent upon tissue type within the DEZ and that the cells in the more basal region of the DEZ are more tightly controlled by auxin than those in the more apical region. We have also found that the response to submergence differs greatly in roots of the wild type and the *rgr1* mutant (mutant strongly promoted,

little effect in the wild type). This suggests the possibility of differential ethylene sensitivity in these two root types. Also during the past year we have improved the data analysis capabilities of our root subsection analyzing software so we can plot time dependent variations in the angle of each section with separate color-coded curves on a color printer. The hardware for the feedback control of individual root subsection orientation is assembled but we are still working on the modifications of software required to complete the control system. As an extension of our proposal to examine potential variations in sensing/response patterns in different subzones of normal versus starch deficient mutants of *Arabidopsis*, we are collaborating with Tim Caspar at DuPont to obtain samples of high starch mutants. This will allow us to compare gravi-sensing and response in tissue specific regions not only for normal and starch deficient roots but also for enhanced starch mutants. During the coming year we will complete the feedback control loop system and obtain the data for altered gravitropism kinetics under conditions of prolonged maximal stimulation of specific tissue subzones.

This research focuses on an analysis of the cellular mechanisms of plant responses to gravity. The research involves the development of new technology for precise measurements of plant growth and orientation. It is expected that this research will lead to a more complete understanding of how plants sense and respond to gravity. Because it is likely that plant responses to gravity share many features in common with responses to other environmental factors (light, temperature, touch) it is expected that advancing our understanding of plant response mechanisms will lead to improvements in optimizing plant growth under a variety of conditions. It is also likely that these advances will enhance our success of growing plants in novel (e.g., space) environments. In additon to these benefits, there is a more general benefit to the research community to be realized from our development of computerized growth analysis. We have begun developing a plant growth imaging web site devoted to automated analysis of plant growth and standardization of plant growth experimental conditions. This is anticipated to provide a means for greatly accelerating the progress of the research community in the understanding of plant growth.

FY96 Publications, Presentations, and Other Accomplishments:

Evans, M.L. Plant gravitational biology: The need for precise environmental control and remote sensing for post-flight analysis. Proceeedings 18th Space Utilization Workshop in Japan. pp 5.1-5.14 (1996).

Evans, M.L. and Ishikawa, H. Cellular specificity of the gravitropic motor response in roots. Planta (in press).

Evans, M.L. and Ishikawa, H. Computer based imaging and analysis of root gravitropism. ASGSB Bulletin, (in press).

Ishikawa, H. and Evans, M.L. Novel software for analysis of root gravitropism: Comparative response patterns of *Arabidopsis* wild type and *axr1* seedlings. Plant Cell and Environ., (in press).

Young, L., Evans, M., Ishikawa, H., Wolverton, C., and Söll, D. Kinetics of the gravitropic response of primary roots of the *rgr1* mutant of *Arabidopsis thaliana*. Plant Physiology 111: Suppl., p. 136, American Society of Plant Physiologists annual meeting, San Antonio, TX (July 1996).

Young, L., Evans, M.L., Ishikawa, H., Wolverton, C., Simmons, C., and Söll, D. "The gravitropic response of primary roots of the *rgr1* mutant of *Arabidopsis thaliana*" in "Plants In Space Biology." Edited by: Suge, H. and Takahashi, H. Tohoku University Press, pp 73-81, 1996.

Young, L.M. and Evans, M.L. Patterns of auxin and abscisic acid movement in the tips of gravistimulated primary roots of maize. Plant Growth Regul., 20, 253-258 (1996).

Young, L.M., Evans, M.L., Ishikawa, H., and Söll, D. Auxin sensitivity and gravitropic responsiveness in roots of the *rgr1* mutant of *Arabidopsis*. ASGSB Bulletin, Vol. 9: pg. 80, National meeting, Washington DC (1996).

Transduction of the Gravity Signal in Roots of Corn

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Funding:

Project Identification: 199-40-57-26
Initial Funding Date: 4/95
FY 1996 Funding: $74,925

Solicitation:
Expiration: 3/98
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 several steps hypothesized to be 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.

Roots of many species grow downward (orthogravitropism) only when illuminated. Previous work suggests that this is a calcium-regulated response and that both calmodulin and calcium/calmodulin-dependent kinases participate in transducing gravity and light stimuli. During the past year we have begun to characterize these kinases using a molecular approach. A maize root cap library was screened for calcium/calmodulin-dependent kinases and a genomic sequence has been obtained for two such calcium/calmodulin-dependent kinase homologs (MCK1, MCK2). These kinase homologs are expressed in root caps, the site of perception for both light and gravity. MCK1 consists of 7265 base pairs and contains 11 exons and 10 introns. MCK1 is expressed constitutively in both light and dark and therefore, it is unlikely that the light directly affects MCK1 expression though the activity of the protein may be affected by light. In cultivars showing light-regulated gravitropism, we hypothesize that MCK1, or a homolog, functions in establishing the auxin asymmetry necessary for orthogravitropism. We also attempted to eliminate (knock-out) calcium-calmodulin activity using maize plants in which genes for this homolog are interrupted, or otherwise non-functional. However, our efforts did not produce a distinct phenotype, leading us to conclude that calcium-calmodulin-dependent kinases are likely family consisting of at least three distinct genes and suggests that if this gene, or a homolog, is involved in gravity signal transduction, that there may be several redundant pathways. Future work will be directed towards

characterizing the protein products of these genes, including producing antibodies to purify the native, endogenous protein.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Feldman, L.J. Evolution of the gravity signal transduction pathway in plants. Astrobiology Workshop, Ames Research Center (1996).

Feldman, L.J. Gravitropism in roots. Gordon Conference: Gravitational Effects on Living Systems (1996).

Feldman, L.J. Light-regulated gravitropism: A role for protein kinases. International Workshop on Plant Biology in Space. Bonn, Germany (1996).

Feldman, L.J. and Lu, Y-T. (abstract) Light-regulated root gravitropism: A role for and characterization of a calcium/calmodulin-dependent protein kinase homolog. ASGSB Bulletin, 10, 72 (1996).

Lu, Y-T. and Feldman, L.J. Characterization of a calcium/calmodulin-dependent protein kinase homolog from maize roots showing light-regulated gravitropism. Planta, 199, 18-24 (1996).

Mechanism of Phytochrome Regulation of Shoot Gravitropism in Arabidopsis

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Funding:

Project Identification: 199-40-57-42
Initial Funding Date: 7/95
FY 1996 Funding: $82,000

Solicitation: 01-13-94/GB
Expiration: 6/97
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.

We previously found that the *Arabidopsis* phytochrome B mutant allele, hy3-1 (also called Bo64), was found to also contain a second site mutation that is responsible for an abnormal gravitropic response that was previously attributed to the phytochrome mutation. The mutation in the gravity response has been tentatively named hgr1 (hypocotyl gravity). Plants containing the hgr1 mutation were isolated from F2 plants derived from a cross between hy3-1 and wild type. The hgr1 mutation has been mapped to a position on chromosome 1 at approximately 20 cM between the physical markers AthGENEA and AthATPase. We are continuing to refine the map location using additional marker genes that are at chromosome position closer to hgr1 in an effort to eventually clone the mutant gene for molecular studies on it potential function in gravitropism in *Arabidopsis*. We have also completed the characterization of the phenotype of the hgr1 mutation in backcrossed lines lacking the hy3-1 mutation. We found that the hypocotyls and roots of hgr1 plants display agravitropic (random

orientation) growth in darkness. After 90 ° reorientation treatments, hgr1 hypocotyls and roots develop minimal gravicurvature compared to wild type. Etiolated seedlings exposed to unilateral blue light showed a stronger phototropic response compared to wild type. Because hgr1 develops phototropic curvature, it indicates that the mutation is specific to the gravitropic signal transduction. Moreover, the increased phototropic curvature is consistent with the idea that phototropism is normally limited by competition with gravitropism. In contrast to the lack of gravitropic response in hypocotyls, inflorescence stems display gravitropism. Upon reorientation by 90°, the inflorescence of hgr1 plants show the beginning of gravicurvature with approximately the same kinetics as wild type. However, time-lapse analysis of inflorescence growth reveal that after the initiation of gravicurvature, the inflorescence undergoes extremely exaggerated nutational movements and it takes several hours before the plants resume vertical growth orientation. Moreover, the nutational movements in vertically growing stems also display a much greater amplitude than wild type. The increased amplitude translates into a greater nutation period such that the hgr1 stems complete a single rotation in about 190 min compared to 120 min for wild type. Time-lapse studies suggest that nutational movements are influenced by gravity since the hgr1 mutation that alters gravitropism also alters nutational movements in the shoot. Moreover, the time-lapse results indicate that graviperception is functional in hgr1 but that the response is abnormal suggesting that hgr1 functions downstream in signal transduction. We also found that unlike several gravity response mutants that are auxin-related, hgr1 shows wild-type sensitivity to IAA and NPA with respect to inhibition of hypocotyl and root elongation.

To identify additional gravity response mutants that are altered in light-dependent effects on their gravity response, we have characterized 16 auxin-and ethylene-related mutants and have identified three that have altered gravitropic behavior and that do not show the red-light-induced changes in gravitropism that we have previously demonstrated to be typical of *Arabidopsis* hypocotyls. These three mutants are axr1-3, axr5, and tir5. In Dr. Estelle's lab, axr1-3 and axr5 were originally isolated as auxin response mutants and tir5 was isolated as an auxin transport inhibitor resistant mutant. axr5 and tir5 completely lack phyA- and phyB-dependent effects on their gravity response but other phyA and phyB responses are the same as in wild type. The axr1-3 mutant shows partial impairment of the phytochrome effect on gravitropism but is normal in other phytochrome responses. These mutants are currently being used to further define how phytochrome alters the way auxin functions during gravitropism. Associated with this work, we have also characterized some unique aspects of the auxin physiology of axr1 and axr2 mutants. Because of its potential involvement in auxin transport, we are focusing our attention on tir5.

We have also made progress on our physiological studies of the interaction between phytochrome and the gravitropic responses of leaves in *Arabidopsis*. In plants grown under different red:far-red ratios, leaves are held at different angles with higher angles at low red:far-red ratios. Changes in leaf angles are also observed when plants were moved between different light conditions. For example, in darkness the leaves become nearly vertically oriented. The magnitude of the dark response depends on the time within the diurnal cycle that the treatment was imposed suggesting involvement of a circadian regulator. When grown under low red:far-red ratios, phya-phyb double mutants exhibited steeper leaf angles relative to wild-type plants, suggesting a role for these phytochromes in controlling this response. Reorientation studies suggest that the phytochrome-dependent control of leaf angle appears to be at least partly due to an effect of phytochrome on the gravitropic response of the leaves. In nature, these phytochrome changes in leaf arrangement may increase the competitive ability of plants growing in dense stands. In addition, these findings suggest that light quality within a plant canopy may determine the angle of branching as well as leaf arrangements, and thus, could be an important regulator of overall plant morphology. We are currently attempting to isolate mutants that display altered leaf orientation under various light conditions and will use the mutants as model systems for investigating the gravitropic responses of lateral organs and their importance to plant development.

In our studies on the interaction of gravitropism and phototropism, we discovered that double mutants that are lacking both phyA and phyB are severely defective in the development of phototropic curvature. When one or the other phytochrome is present, the plants display significant blue light-dependent phototropic curvature. Because phototropism is considered to be a blue-light-specific response, our results were rather surprising. However, our current results suggest that full phototropic competence requires phytochrome to alter the

gravitropic response of the hypocotyl and to increase the sensitivity to blue light. Preliminary analysis of the individual phytochrome mutants suggests that phyA may be primarily responsible for increasing blue light sensitivity while phyB may be more important for altering the gravitropic response.

These investigations on the interactions of gravity responses and the different photosensory systems in *Arabidopsis* are providing insights into the nature of the complex network of sensory response systems that regulate plant development. The gravitropic response system is clearly a central component of this environmental sensory network.

Plant morphology is an important agronomic trait that affects plant productivity. For example, branching patterns can affect overall photosynthetic capacity of a plant and, thus, alter yield. In addition, the angle of branch growth can affect spacing of plants and impact planting density. Because gravitropism affects these and many other aspects of plant growth, understanding how gravity helps shape a plant into its final form is not only of fundamental importance for understanding plant growth and development but may have important agronomic implications. Our discovery that different photosensory systems modulate the gravitropic responses in aerial parts of plants suggest that it may be possible to engineer plants that will display growth habits that are suitable to a wider range of growth practices than are currently available. For example, since genes for the various phytochromes have been cloned, it is possible to change the levels of specific phytochromes in specific organs of a transgenic plant. By understanding how the different phytochromes affect gravitropism and thus affect branch angles, it should be possible to use the information from our research to improve yield potential for some crops. For example, by modifying the ratio of phyA and phyB in branches, it may be possible to construct a plant that will have more upright branches and allow closer planting while maintaining a high photosynthetic capacity and possibly resulting in higher yields.

FY96 Publications, Presentations, and Other Accomplishments:

Hangarter, R.P. Interactions between blue light, phytochrome and gravity response systems in *Arabidopsis*. International Meeting on UV/Blue light Perception and Responses in Plants and Microorganisms. Philipps-Universität, Marburg, Germany, August 1996.

Hangarter, R.P. Interactions of phytochrome, gravitropism and phototropism in *Arabidopsis*. Gordon Research Conference on Gravitational Effects on Living Systems, Colby-Sawyer College, New London, NH, July 1996.

Hangarter, R.P. Phytochrome regulation of gravitropsim: Integration of environmental sensory systems. NSCORT Symposium on Calcium and Gravitational Biology, North Carolina State University, November 1996.

Poppe, C., Hangarter, R.P., Sharrock, R.A., Nagy, F., and Schäfer, E. The light-induced reduction of the gravitropic growth-orientation of seedlings of *Arabidopsis thaliana* (L.) Heynh is a photomorphogenic response mediated synergistically by the Pfr-forms of phytochrome A and B. Planta, 199, 511-514 (1996).

Interaction of Light and Ethylene in Stem Gravitropism

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Funding:

Project Identification: 199-40-57-36
Solicitation: 12-10&11-92/GB
Initial Funding Date: 10/92
Expiration: 9/95
FY 1996 Funding: $0
Students Funded Under Research: 7

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

We are investigating 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 biosynthesis of the plant hormone ethylene during gravitropic bending in etiolated pea stems. Previous investigations indicated that ethylene production increases during gravitropic curvature in stem tissue. This increase is thought to occur during the later phases of bending in etiolated stems when the curving zone migrates downward, the tip region straightens, and overall curvature slows. Both red-light illumination and ethylene treatment alter the kinetics and locus of curvature. Specifically, red-light illumination reduces curvature migration resulting in a more constant rate in a localized area of the stem.

This project ended in 1996, and this final report summarizes the results on the regulation of ethylene biosynthesis during gravitropic curvature in the presence or absence of red-light treatment. Specifically, the following questions were addressed: (1) Which regulatory step in ethylene biosynthesis is primarily affected by a change in stem orientation? and (2) Which regulatory step is affected by red-light treatment? For dark-grown pea stems, the tissue levels of the ethylene precursor ACC transiently increased by 30 min gravistimulation. The

level of conjugated ACC before gravistimulation was significantly higher in etiolated pea stems compared to red-pretreated seedlings. This high level of conjugation declined after gravistimulation and may account for the transitory increase in ACC observed at 30 min. No change in ACC oxidase activity was observed during gravitropic bending in etiolated stem tissue. Thus, a slight change in ethylene biosynthesis during gravitropic curvature is most likely due to a transitory change in ACC conjugation level producing excess ACC. Seedlings pretreated (18 hours before experimentation) with red-light exhibited increased ACC levels during the later phases of curvature (90 and 120 min gravistimulation). However, no significant differences in ACC levels between upper and lower stem tissue were found. The overall level of ACC conjugation and oxidation did not change in red-light treated tissue throughout the time course of gravitropic curvature. Thus, red light regulation of ethylene biosynthesis by ACC (via ACC synthase) is a primary focus of our continuing research.

For studies involving microscopic analysis, 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. Peroxidase activity (as a model system) was evaluated using microscopy and computer image analysis of cross- and longitudinal-sections of pea stems imprinted on nitrocellulose. DNA probes for ACC synthase mRNA with colorimetric conjugates were developed for this system. Because of the low level of ACC synthase in pea tissue, it was not detectable by this procedure. Fluorescent markers and confocal microscopy are currently being examined to determine the feasibility of evaluating tissue localization of ACC synthase transcript levels by *in situ* hybridization.

The changes in peroxidase and invertase levels during gravitropic bending in etiolated and red-treated pea stems were investigated by spectrophotometry and isoelectric focusing. In etiolated tissues, there was a slight increase in peroxidase specific activity in the upper stem during gravitropic bending. At 60 min gravistimulation, the upper tissue also exhibited a novel peroxidase isozyme at pI 4.2 and increased band density for the isozyme at pI 8.3. Invertase activity in etiolated stems increased by 60 min gravistimulation and was evenly distributed in the upper and lower portions of the stem. However, invertase specific activity decreased to its initial level by 120 min gravistimulation. In red-pretreated tissue, peroxidase activity increased by 30 min gravistimulation and remained high throughout the time course of gravitropic curvature. This increase was accompanied by higher protein content so that the specific activity of peroxidase increased only at 120 min gravistimulation. There were no novel peroxidase isozymes noted for the red-treated tissue. The isoelectric-focusing gels indicated that the peroxidase isozyme at pI 8.3 increased uniformly after gravistimulation. Invertase activity in red-pretreated stem tissue also increased after gravistimulation primarily during the later phases of curvature and in the lower side of the stem. In conclusion, protein content, invertase activity, and some peroxidase activity increased during rapid upward curvature (30 to 60 min gravistimulation) in etiolated stems. This may reflect the increased cell growth and cell wall synthesis during these times. Red-light pretreatment also caused enzymatic changes primarily during the later phases of gravitropic curvature. These changes may also be attributed to rapid curvature at this time since the red-light pretreated stems do not exhibit migration of curvature and net slowing as do etiolated stems.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Steed, C.L. and Harrison, M.A. Interaction of light and ethylene on gravitropism in etiolated pea stems. ASGSB Bull., 10, 40 (1996).

Magnetophoretic Induction of Root Curvature

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Funding:

Project Identification: 199-40-57-37
Solicitation:

Initial Funding Date: 5/95
Expiration: 4/96

FY 1996 Funding: $0
Students Funded Under Research: 2

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

The goal of this project 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.

The objective of the previous research was a detailed analysis of curvature induction in roots and intracellular magnetophoresis of amyloplasts. Based on the information from these studies we examined whether positively gravitropic shoot curvature could be induced by high gradient magnetic fields (HGMF), an objective that had been attempted earlier but was not achieved, presumably because of insufficiently small magnetic gradients.

We tested coleoptiles (oat, *Avena sativa* and barley, *Hordeum vulgare*) by positioning them in a HGMF. The dynamic factor °H2/2 of the field was 109 to 1010 Oe2/cm. The HGMF was generated by inserting a ferromagnetic wedge into a uniform magnetic field (ca. 4.5 kOe). To minimize gravity effects the seedlings and magnets were rotated on a 1-rpm clinostat. After 4 hours, 90% of coleoptiles had curved toward the denser HGMF, i.e. had behaved as predicted for negatively gravitropic organs. Coleoptiles in a magnetic field next to a non-ferromagnetic wedge (i.e. no HGMF), showed no preferential curvature. The small size of the area of non-uniformity of the HGMF allowed mapping of the sensitivity of the coleoptiles by varying the initial position of the wedge relative to the coleoptile apex. When the ferromagnetic wedge was placed lower than

1mm below the coleoptile tip only 58% of the coleoptiles curved toward the wedge indicating that the cells most sensitive to intracellular displacement of amyloplasts, and thus gravity sensing, are confined to the top 1 mm portion of barley coleoptiles. Similar experiments with tomato (*Lycopersicum esculentum*) hypocotyls also resulted in curvature toward the HGMF.

Based on these and previous results we now have strong support for the amyloplast-based gravity sensing system in higher plants. A second accomplishment is the usability of HGMF to substitute gravity in roots and shoots under microgravity conditions. Future investigations, partially dependent on a shuttle experiment scheduled for 1998, focus on several objectives: Does microgravity alter the density of starch and do plants acquire higher or lower gravi- or magnetophoretic sensitivity.

The observation that amyloplasts in the periphery of the root cap cells do not sediment upon gravistimulation or move due to magnetophoretic forces suggests that the cytoskeleton fixes these organelles in place. We will study the magnitude of the forces exerted by the cytoskeleton and which component of the cytoskeleton (actin filaments or microtubules) contributes to the anchoring of amyloplasts and possibly other organelles.

The application of high gradient magnetic fields to investigate the gravity sensing mechanism of plants has wide implications on two levels. First, the research utilizes and improves a novel mechanism of intracellular displacement of starch filled amyloplasts and the resulting growth response of plants. Secondly, the growth response is likely to be tightly linked to the perception of a stimulus analogous to gravity. Therefore the research addresses the larger problem of studying the signal perception/response mechanism in plants. Such studies will generally promote our understanding of plant growth regulation. In particular, the high gradient magnetic field-dependent growth response will elucidate the change in elongation growth and thus directional deposition of cell wall material biomass. In addition, as with all basic research, an improved understanding of basic growth phenomena will have important implications for improving growth, biomass production on earth, and better understanding of the biomechanic properties of growing plants. Thus, this research will benefit the average citizen.

FY96 Publications, Presentations, and Other Accomplishments:

Hasenstein, K.H., Kuznetsov, O.A., and Blancaflor, E.B. Induction of plant curvature by magnetophoresis and cytoskeletal changes during root graviresponse. 6th Europ. Symp. on Life Sci. Res. in Space, ESA SP-390: 71-74.

Kuznetsov, O.A. and Hasenstein, K.H. Magnetophoretic induction of root curvature. Planta, 198, 87-94 (1996).

Wan, Y. and Hasenstein, K.H. Preparation and Characterization of Anti-idiotypic antibody to probe putative abscisic acid receptors. Intern. J. BioChrom., 2, 77-88 (1996).

Self-Generating Bending Moments in Root Gravitropism

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Project Identification: 199-40-57-35
Solicitation:
Initial Funding Date: 6/93
Expiration: 9/96
FY 1996 Funding: $0
Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

Task Description:

The main direction of this project remains unchanged. We are still attempting to describe the principal biomechanical parameters of seedling germination. In particular, we are trying to determine the levels of force output of gravitropically responding corn roots. In addition, we are investigating the effects of tip loading on germinating roots. Within the general thrust of this project, however, a new direction has emerged. In our effort to determine the biomechanical parameters surrounding root growth we have come up with a novel method for the determination of turgor pressures within plant cells. Our method, which is unique in its rapidity and repeatability, 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 zero 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.

Fourth, our studies of root gravitational sensitivity in corn has yielded exciting new information regarding the specific location of the gravity sensor. We have determined that the gravitational sensitivity of corn roots is mediated by a cell surface receptor analogous to the integrin receptors in animal cells.

This project will continue to yield new understanding of the basic biological process of seed germination and seedling establishment. During the first hours after the emergence of the root from the dormant seed, the root must first determine the direction of the gravity vector; second it must actively bend towards the substrate, (the earth), and third, it must successfully penetrate the earth in order to establish a viable seedling. This research will yield a better understanding of these critical biomechanical processes, enhancing our ability to understand and manipulate the germination process both on Earth and in space, where the principal cue for these processes, namely the gravity vector, may be severely attenuated or lacking. Eventually, this work could be translated into modified agricultural practices and improved germination rates based on a better understanding of the basic biomechanical parameters underlying seedling performance during germination. Immediate benefits include the development of a new technology for the rapid, non-destructive measurement of cell turgor pressures, an essential measure of water stress, and a critical element in the developmental mechanics of plant growth.

FY96 Publications, Presentations, and Other Accomplishments:

Kuzeja, P. and Lintilhac, P.M. Mechanical performance of corn roots under tip load. American Society for Gravitational and Space Biology, Crystal City, VA (October 26, 1995).

Lintilhac, P.M. and Lynch, T. Plant cell division under mechanical stress: The importance of strain-field geometry. American Society for Gravitational and Space Biology, Crystal City, VA, (October 26, 1995).

Lynch, T.M. and Lintilhac, P.M. Mechanical signals in plant development: A new method for single cell studies. Developmental Biology, (in press).

Lynch, T.M. and Lintilhac, P.M. First place award for paper entitled "Plant cell division under mechanical stress: The importance of strain-field geometry". Annual Meeting American Society for Gravitational and Space Biology, Crystal City, VA (October 25-29, 1995).

Outwater, J.O. and Lintilhac, P.M. A rapid non-invasive method for measuring turgor pressure in exposed plant cells. American Society for Gravitational and Space Biology, Crystal City, VA (October 28, 1995).

Gravitropic Signal Transduction in the Lazy-2 Tomato Mutant

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Funding:

Project Identification: 199-40-57-45
Solicitation: 95-OLMSA-01
Initial Funding Date: 7/96
Expiration: 6/99
FY 1996 Funding: $118,915.00
Students Funded Under Research:

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 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 supressors of that mutation. During the current grant period, we have also begun genetic studies designed to map the lazy-2 lesion. These efforts will be continued and should result in map-based cloning of the mutated gene. This will provide an important link between red light and control of stem elongation and help to elucidate the mechanism of the plant gravitropic response. The research supports the goals of the Space Biology Program in determining the effects of the interaction of gravity and another environmental factor (red light) on biological systems. A better understanding of the lazy-2 mutation should lead to well-defined flight experiments which will test the possibility that proper light manipulations can compensate for the absence of gravity in regulating stem development and orientation.

Information regarding specific progress made during FY96 was not provided by the principal investigator.

Molecular Cloning of the *Arabidopsis thaliana* AGR1 Locus

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Project Identification: 199-40-57-46
Initial Funding Date: 3/96
FY 1996 Funding: $129,902

Solicitation: 95-OLMSA-01
Expiration: 2/99
Students Funded Under Research: 4

Task Description:

Plants orient the growth of their primary organs according to the gravity vector. In roots, a gravity stimulus induces a reorientation of growth in the elongation zone, provided the root cap is present. The long-term objectives of our research are to define the molecular events involved in gravity sensing and signal transduction by the root of *Arabidopsis thaliana*.

We have identified several *A. thaliana* mutants affected in root gravitropism. Two of them are likely to be affected in gravity sensing and/or early phases of gravity signal transduction. *agr1* mutants are characterized by a specific defect in root gravitropism and ethylene resistance, while *arg1* mutants present a defect in both root and hypocotyl gravitropism. Unlike most other agravitropic mutants, *agr1* and *arg1* mutants show no pleiotropic phenotypes. *agr1* was mapped on the South arm of chromosome V, while *arg1* was localized to the South arm of chromosome I. The present proposal is aimed at cloning and characterizing molecularly the *agr1* and *arg1* loci, using a combination of chromosome walking and transposon tagging strategies.

To better characterize the molecular functions of these loci, we will also clone and sequence the corresponding cDNAs and mutant alleles of both loci, and we will characterize the corresponding proteins. Also, we will characterize the pattern of expression of both genes, and we will localize the corresponding proteins within the plant cells and organs. The genetic, physiological and molecular characterization of these mutations should provide important information expanding our understanding of the molecular mechanisms underlying gravity sensing and transduction in plant roots.

The general objectives of our research are to understand the molecular mechanisms by which plant organs sense a change in their orientation within the gravity field and transduce that physical information into a physiological response resulting in a change of growth direction by the stimulated organ. The specific aims of this project are to clone and characterize molecularly two *Arabidopsis thaliana* genes involved in this process: *agr1* and *arg1*. Mutations in these genes result in altered root gravitropism without concomitant increases in resistance to exogenous auxin, a growth regulator known to regulate the gravity-induced reorientation response (Masson, 1995).

agr1 mutant seedlings are specifically altered in root gravitropism (Bell and Maher, 1990; Masson et al., 1993). We have shown that some *agr1* alleles also confer an increased resistance to exogenous ethylene, suggesting a

role for ethylene in root gravitropism. We have further shown that *agr1* is allelic to *eir1*, a mutant isolated by the Ecker laboratory for its defect in root gravitropism and resistance to exogenous ethylene (Roman et al., 1995). A chromosome walking strategy was initiated to clone *agr1*. A YAC clone carrying the locus was identified, and two polymorphic loci were mapped at 0.1 and 4 cM of *agr1*, flanking it. We have constructed a small contig of genomic DNA fragments in lambda and cosmid vectors overlapping with the closest marker, and we are investigating the location of *agr1* on that contig (Hilson, Chen, and Masson, unpublished data). This chromosome walking strategy, now close to completion, should elucidate the molecular structure of *agr1*, a prerequisite for a better understanding of its role in the gravity and ethylene signal transduction pathways.

arg1 mutant seedlings develop abnormal root and hypocotyl gravitropism. Interestingly, they show no other pleiotropic phenotypes, suggesting that *arg1* is involved in gravity sensing and/or early phases of gravity signal transduction (Masson et al., 1993; Masson, 1995). We have mapped *arg1* on the South arm of chromosome 1 (Sedbrook and Masson, in preparation), on a contig of cloned genomic DNA sequences in a YAC vector (Vijayraghavan et al., 1995). Using cloned polymorphic sequences covering that region of chromosome 1 (kindly provided by Dr. Vijayraghavan), we have identified a YAC genomic fragment carrying the locus. We then mapped *arg1* on a 35 kb piece of genomic DNA derived from that YAC. That fragment was subcloned, and each subfragment was tested for its ability to complement the *arg1* mutation, upon transformation. We have identified and sequenced a 9 kb DNA subfragment able to complement *arg1*. A candidate sequence for *arg1* has been identified on that fragment, and we are sequencing the two mutant alleles currently available at that locus (*arg1-1* and *arg1-2*) to demonstrate its identity. cDNAs hybridizing with the 9 kb genomic DNA fragment have also been identified and are being sequenced and characterized (Sedbrook and Masson, in preparation). Additionally, experiments are in progress to define the pattern of expression under gravity induced and uninduced conditions, using a combination of Northern blot analysis, *in situ* hybridization, and the production of transgenic plants expressing *arg1p-GUS* and *arg1p-GFP* reporter genes (Sedbrook and Masson, in preparation). In the future, we will overproduce the *arg1* protein in *E. coli* and use the purified protein to direct antibodies against it. These antibodies will be used to characterize the cellular location of the *arg1* protein, to define the regulation of *arg1* expression, and to study possible post-translational modifications of the protein.

This combination of genetic, molecular, and physiological analysis of the *agr1* and *arg1* loci should allow us to characterize the function(s) of both genes and, consequently, to better understand specific events in the signal transduction pathway resulting in the gravitropic response of plant organs.

Plant organs use the gravity vector as a cue to define the vector of their growth. That response, named gravitropism, allows for shoots to grow upward and for roots to grow downward. This is of major interest for agricultural productions as it allows crop plants to orient their organs optimally for photosynthesis and for water and nutrients uptake. It also allows crop shoots to resume vertical growth after being prostrated by the action of wind and rain, thereby maintaining seeds away from soil moisture and available for mechanical harvest.

FY96 Publications, Presentations, and Other Accomplishments:

Masson, P.H. Molecular genetic analysis of root gravitropism and waving in *Arabidopsis thaliana*. NSF/Japanese workshop on Plant Signal transduction Tsukuba, Japan (May 8-9, 1996).

Masson, P.H. Presentation entitled: Characterization of the AGR1 and ARG1 loci involved in root gravitropism in *Arabidopsis thaliana*. FASEB Meeting, Colorado (June 16-21, 1996).

Masson, P.H. Root gravitropism and waving in *Arabidopsis thaliana*. University of Wisconsin-Madison, Laboratory of Genetics (October 31, 1996).

Masson, P.H. Root gravitropism and waving in *Arabiodopsis thaliana*. Marquette University, Milwaukee (November 22, 1996).

Rutherford, R. and Masson, P.H. *Arabidopsis thaliana sku* mutant seedlings show exaggerated surface-dependent alteration in root growth vector. Plant Physiol., 111, 987-998 (1996).

Rutherford, R. and Masson, P.H. (poster) Patterns of *Arabidopsis thaliana* root growth on agar surfaces. 7th International Conference on Arabidopsis Research, Norwich, UK (June 23-27, 1996).

Sedbrook, J.C., Kronebusch, P.J., Borisy, G.G., Trewavas, A.J., and Masson, P.H. Transgenic *AEQUORIN* reveals organ-specific cytosolic Ca^{2+} responses to anoxia in *Arabidopsis thaliana* seedlings. Plant Physiol., 111, 243-257 (1996).

Sedbrook, J., Caspar, T., and Masson, P.H. (poster) Phenotypic characterization and mapping of agravitropic mutations in the ARG1 locus of *Arabidopsis thaliana*. Gordon Research Conference on Gravitational Effects on Living Systems, Colby-Sawyer College, New-London, NH (July 15-19, 1996).

Suh, L. and Masson, P.H. Isolation and characterization of a RNA transcript that is upregulated in maize root tip upon gravistimulation. Gordon Research Conference on Gravitational Effects on Living Systems, Colby-Sawyer College, New-London, NH (July 15-19, 1996).

Molecular Genetics of Root Thigmoresponsiveness in Arabidopsis thaliana

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Funding:

Project Identification: 199-40-57-39
Solicitation: 01-13-94/GB
Initial Funding Date: 4/95
Expiration: 3/97
FY 1996 Funding: $147,092
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.

When *Arabidopsis thaliana* seedlings grow on the surface of an agar medium tilted forward at some angle from the vertical, their roots develop a wavy pattern of growth (Okada and Shimura, 1990; Rutherford and Masson, 1996). Last year, we showed that root waving involves a succession of left-handed and right-handed circumnutation-like processes and derives from a combination of responses to gravistimulation, touch, and probably other surface-derived stimuli. To better define the molecular mechanisms involved in the regulation of this process, we have identified and initiated the characterization of mutants developing altered root waves under these conditions.

wvc1 mutant roots develop compressed, square-like waves on titled agar surfaces. Last year, we showed that *wvc1* derives from the insertion of a T-DNA in the *Arabidopsis ASA1* locus, a redundant gene which codes for one of the two subunits of anthranilate synthase, an enzyme involved in tryptophan (TRP) biosynthesis. In plants, the TRP biosynthesis pathway is utilized for the production of a number of secondary compounds with important biological functions, including several auxins, phytoalexins, and indole-glucosynolates (Niyogi and Fink, 1992). Using a combination of Northern and Western blot analysis, we were able to show that *wvc1* is most likely a null mutation of the *ASA1* locus.

ASA1 function is redundant with that of *ASA2*. *ASA2* is expressed constitutively at a low level in most organs of the plant, while *ASA1* is constitutively expressed at a higher level and is further upregulated by wounding and by compatible plant-pathogen interactions (Niyogi and Fink, 1992). Quite surprisingly, we found that wild type and *wvc1* mutant seedlings contain equal levels of free TRP under uninduced conditions. However, when seedlings were wounded, the level of free TRP decreased in the mutant relative to the wild type, suggesting that the activation of a secondary branch of the biosynthetic pathway uses up some intermediate(s) in the main pathway, resulting in a decrease in the biosynthesis of TRP (Rutherford, Gallois, and Masson, in preparation).

To better define the involvement of the TRP biosynthesis pathway in root waving, we have attempted to rescue the phenotype by adding various intermediates and end-products of that pathway to the medium. Anthranilate and L-TRP rescued the phenotype, while indole-3-acetic acid (IAA), indole-3- butyric acid (IBA), or D-TRP did not. Preliminary results also indicate that indole-3-lactic acid (ILA), a secondary product derived from TRP in that pathway, is capable of dampening the waves of both wild type and *wvc1* mutant roots when added to the agar medium. These results suggest that ILA itself, or a compound derived from ILA, is an important regulator of the circumnutation-like process which accompanies root waving. If that hypothesis is correct, these results also suggest that the biosynthesis of that compound is regulated at a step following TRP in the pathway. However, it should be emphasized that we have not yet eliminated the possibility that the effect of ILA on root waving is nonspecific (Rutherford and Masson, in preparation), nor have we excluded the possibility that the compressed root wave phenotype of *wvc1* seedlings results from a localized TRP deficit in the tip of waving roots, limiting the biosynthesis of TRP-rich protein(s) required for the development of wild type waves.

We have also initiated the characterization of another mutant with compressed root waves on tilted agar surfaces. *wvc16* was identified in a collection of *Ds* mutagenized plants. Preliminary results indicate that *wvc16* is tagged by a *Ds* insert, and we have cloned some of the genomic sequences flanking that insertion. We have also characterized the phenotype further and found that mutant shoots carry curled leaves, twisted and coiling stems, and curly siliques. This phenotype, although not totally penetrant, is consistent with the root phenotype described above, suggesting that *wvc16* promotes a structural change resulting in increased coiling of developing organs. The molecular analysis of this gene, currently in progress, should provide important clues on the structural basis for the circumnutation-like process which accompanies root waving in *Arabidopsis thaliana*.

While mutants developing compressed root waves on tilted agar surfaces are likely to allow the identification of important molecules involved in the regulation of root waving, mutants developing dampened or no waves under these conditions are likely to reveal essential players in that complex growth response. We have identified six mutants developing dampened root waves on tilted agar surfaces. Most of these mutants were derived from collections of *T-DNA*- or *Ds*-tagged lines. A careful genetic analysis indicates that at least 3 of these mutations are semi-dominant. Cosegregation analysis of one of these mutations (*wvd2*) with the *Ds* insertional mutagen used to generate the corresponding collection indicates that it is tagged by a *Ds* insert. Interestingly, the *wvd2* mutation also confers a left slanting root growth phenotype on vertical agar surfaces, in contrast with the right slanting root growth phenotype exhibited by wild type plants of the same ecotype (Pearlman, Suh, and Masson, unpublished; Rutherford and Masson, 1996). We have cloned genomic sequences flanking *Ds*, and we are characterizing the corresponding wild type gene. A structural analysis of the locus, along with a characterization of its pattern of expression in wild type plants and the determination of the location of the corresponding protein in wild type and various mutant plants should allow us to better understand the involvement of that locus in root waving and skewing on agar surfaces. Additionally, the molecular characterization of other dampened root wave mutants should allow us to better understand the molecular mechanisms involved in root waving.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Masson, P.H. Molecular genetic analysis of root gravitropism and waving in *Arabidopsis thaliana*. NSF/Japanese workshop on plant signal transduction Tsukuba, Japan (May 8-9, 1996).

Rutherford, R. and Masson, P.H. *Arabidopsis thaliana sku* mutant seedlings show exaggerated surface-dependent alteration in root growth vector. Plant Phyiol., 111, 987-998 (1996).

Sedbrook, J.C., Kronebusch, P.J., Borisy, G.G., Trewavas, A.J., and Masson, P.H. Transgenic *AEQUORIN* reveals organ-specific cytosolic Ca^{2+} responses to anoxia in *Arabidopsis thaliana* seedlings. Plant Physiol., 111, 243-257 (1996).

The Role of Actin Cytoskeleton in Auxin Transport and Gravitropism

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Funding:

Project Identification: 199-40-57-41
Initial Funding Date: 4/95
FY 1996 Funding: $83,228

Solicitation: 01-13-94/GB
Expiration: 3/98
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 growth and gravitropism. This year, a study designed to determine if the NPA binding protein (NBP) of the auxin efflux carrier is specifically associated with actin was completed, and a publication from this study is currently under review. This work was performed by a NASA supported

graduate student and technician. This study resulted in both *in vitro* and *in vivo* evidence linking auxin transport to the actin cytoskeleton. Another graduate student, with partial NASA support, has completed a thesis which suggests that the cytoskeletal association of the NBP is reduced under conditions where auxin transport is reduced, such as when ethylene concentrations are elevated. The work in this thesis is now being edited for publication. An undergraduate in the laboratory initiated a study to ask about the functional role of the two streams of polar auxin transport in roots. These results indicate that auxin moving from the shoot into the root controls lateral root development, while auxin moving from the root tip toward the shoot controls gravity response, growth, and root waving. This work was completed by a NASA supported technician, and two manuscripts resulted from this work, one of which is in review and the other of which is in preparation. Additionally, another NASA supported graduate student has modified our procedures for measuring NPA binding activity and auxin transport for use with *Arabidopsis thaliana*. These assays will now be used to examine auxin transport in mutants altered in gravitropism and auxin transport inhibitor sensitivity. One such analysis, in collaboration with Dr. Mark Estelle, is currently in press at the Plant Cell. Finally, although our original plan to examine the relationship between auxin transport and the actin cytoskeleton was to use Characean algae, we have altered our plans to ask these questions using the brown algae *Fucus* as a model system. An undergraduate in the lab found that gravity affects *Fucus* growth and development in two ways. The angle of the gravity vector determines the site of development of polarity in zygotes of this plant, and newly initiated rhizoids will bend to grow in the gravity vector, much as roots of higher land plants. The gravity dependent development of *Fucus* zygotes was found to depend upon the actin cytoskeleton, since fragmentation of actin filaments with cytochalasin D prevents gravity dependent development.

The goal of the research supported by this grant is to increase understanding of a basic biological process, the response of plants to gravity. This study focuses on the role of transport of a class of plant hormones, the auxins, in gravity response. The long-term goals of this study include understanding how one of the proteins which control auxin transport functions and how gravity stimuli may affect this protein's function to allow changes in auxin transport and changes in growth in response to gravity. A clearer understanding of the mechanisms by which plants respond to changes in the vector of gravity may provide important insight into predicting how plants will grow in the absence of gravity and may facilitate the design of experiments to study plant response during space flight.

FY96 Publications, Presentations, and Other Accomplishments:

Brady, S.R., Dixon, M.W., Cyr, R.J., Fisher, D.D., and Muday, G.K. (abstract) BY2 Protoplasts as a Model for NPA Sensitive Auxin Transport and Gravitropism. ASGSB Bull. 10, 78 (1996).

Brown, D.E., Reed, R.C., and Muday, G.K. (abstract) Polar Auxin Transport and Gravitropism in *Arabidopsis Thaliana*. Grav. and Space Biol. Bull. 10, 38 (1996).

Hu, S., Dixon, M.L., and Muday, G.K. (abstract) Development of a Procedure to Depolymerize the Actin Cytoskeleton for Purification of Associated Proteins. ASGSB Bull. 10, 27 (1996).

Luciano, R.L. and Muday, G.K. (abstract) Characterization of Gravity Dependent Growth in *Fucus* Zygotes. ASGSB Bull. 10, 26. (1996).

Muday, G.K. Actin association of an auxin transport protein: *In vitro* and *in vivo* evidence. Federation of the American Societies for Experimental Biology summer meeting: Plant Signal Transduction, Copper Mountain, CO.

Muday, G.K. Actin Association of the Naphthylphthalamic acid Binding Protein from Zucchini Hypocotyls. American Society of Gravitational and Space Biology Meeting, Charlotte, NC (October 1996).

Muday, G.K. Association of an Auxin Transport Protein with the Actin Cytoskeleton. Virginia Tech., Interdisciplinary Plant Sciences Seminar, Blacksburg, VA, (February 1, 1996).

Muday, G.K. The Association of an Auxin Transport Protein with the Actin Cytoskeleton. Pennsylvania State University, Biology Department, University Park, PA (January 16, 1996).

Reed, R.C. and Muday, G.K. (abstract) *Arabidopsis* Lateral Root Development Requires Auxin from the Shoot. Plant Phys. 111S, 590 (1996).

Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G., and Estelle, M. Reduced NPA-binding in the tir3 mutant of *Arabidopsis* is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell, (in press).

Microgravity Effects on Early Reproductive Development in Plants

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Funding:

Project Identification: 199-40-57-24
Solicitation: 01-13-94/GB
Initial Funding Date: 10/95
Expiration: 6/96
FY 1996 Funding: $63,157
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.

Work completed during this cycle demonstrated a control of embryo development in *Arabidopsis* by oxygen. Plants were grown full term in pre-mixed atmospheres with oxygen treatments of 2.5%, 5%, 10%, 16%, and 21% oxygen, 350 ppm CO_2, and the balance nitrogen. Seeds were harvested for germination tests and microscopy when siliques had yellowed. Light and scanning electron microscopic observation of nongerminated seeds showed that these embryos had stopped growing at different developmental stages depending upon prevailing oxygen levels. Tissue degeneration caused by cell autolysis and changes in cell structure were observed in cotyledons and radicles. The results demonstrate control of embryo development by oxygen in *Arabidopsis*. Studies with microscopic oxygen electrodes allowed measurement of oxygen inside the locule of developing siliques and confirmed the high oxygen demand by developing seeds. In microgravity, lack of convective air movement may result in stagnation of gases around plant parts. Rapidly respiring tissues such as developing seeds may be at risk in such an environment if diffusion alone cannot meet the gas resupply needs of the plants.

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 at 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.

FY96 Publications, Presentations, and Other Accomplishments:

The Halstead Young Investigator Award was earned by Mary E. Musgrave for space biology research acomplishments (October 1995).

Crispi, M.L., Porterfield, D.M., Murgia, M., and Musgrave, M.E. Role of metabolic gases in reproductive failure under spaceflight conditions: ground based studies with *Arabidopsis*. SAE Technical Paper Series #961391 (1996).

Kuang, A. and Musgrave, M.E. Dynamics of vegetative cytoplasm during generative cell formation and pollen maturation in *Arabidopsis thaliana*. Protoplasma, 194, 81-90 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions. Annals of Botany, 78, 343-351 (1996).

Kuang, A., Xiao, Y., and Musgrave, M.E. (abstract) Anatomy and cytochemical localization of reserves during seed development in *Arabidopsis* under spaceflight conditions. Plant Physiol. Suppl. 111: 73 (1996).

Porterfield, D.M., Crispi, M.L., and Musgrave, M.E. (abstract) Metabolic responses of *Arabidopsis* to altered atmospheres. Plant Physiol. Suppl. 111: 71 (1996).

Porterfield, D.M., Musgrave, M.E., and Dreschel, T. (abstract) Rootzone morphology and alcohol dehyrogenase activity of dwarf wheat grown on nutrient delivery systems designed for microgravity application. Plant Physiol. (Suppl.) 108: 148 (1996).

Mechanotransduction and the Cortical Cytoskeleton: What is the relationship?

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Project Identification: 199-40-57-08
Solicitation:

Initial Funding Date: 3/95
Expiration: 2/96

FY 1996 Funding: $0
Students Funded Under Research: 4

NOTE: An FY 1996 Funding amount of $0 does not necessarily represent zero money spent on the task for FY96. FY 1996 Funding amounts represent task dollars appropriated from the FY96 NASA budget.

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 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/NASFNET 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 postdoctoral fellow in learning new computer techniques, maintaining fragile equipment, and so on.

Though the co-authors of the 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 similar to those that make up the known group of 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 heretofore 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 published in the international journal *Protoplasma*. The contribution to this paper by NAGW-3046 (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 chanels (see review article on *Protoplasma* 182:1-9), and they and the endomembrane sheath are presumed important for the internal signalling 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.

FY96 Publications, Presentations, and Other Accomplishments:

Gens, J.S., McNally, J.G., and Pickard, B.G. (abstract) Fast punctate release of H_2O_2 due to mechanical stimulation of tobacco cells. Plant Physiol., 111 suppl.:813 (1996).

Gens, J.S., Reuzea, C., Doolittle, K.W., McNally, J.S., and Pickard, B.G. Covisualization by computational optical-sectioning microscopy of integrin and associated proteins at the cell membrane of living onion protoplasts. Protoplasma, 194, 2150-2230 (1996).

Calcium Messenger System in Gravitropic Response in Plants

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Initial Funding Date: 7/95
FY 1996 Funding: $30,000

Solicitation:
Expiration: 6/96
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 one-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) were 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.

FY96 Publications, Presentations, and Other Accomplishments:

Poovaiah, B.W. and Reddy, A.S.N. "Calcium and gravitropism" in "Plant Roots: The Hidden Half." Edited by: Waisel, Y., Eshel, A., and Kafkafi, U. Marcel Dekker, Inc./New York, NY, Second Edition, pp 307-321, (1996).

Poovaiah, B.W., Takezawa, D., An, G., and Han, T-J. Regulated expression of a calmodulin isoform alters growth and development in potato. J. Plant Physiol., 149, 553-558 (1996).

Patent Approved, U.S. Patent #: 5,498,533 Poovaiah, B.W., Takezawa, D., Han, T-J., and An, G. "Control of growth and development of potato plants."

Poovaiah, B.W., Wang, W., Takezawa, KD., and Liu, Z.H. Cloning and characterization of genes encoding calmodulin-binding proteins. ASGSB Bulletin 10, 55, (1996).

Takezawa, D., Patil, S., Bhatia, A., and Poovaiah, B.W. Calcium-dependent protein kinase genes in corn roots. J. Plant Physiol., 149, 329-335 (1996).

Takezawa, D., Patil, S., Bhatia, A., and Poovaiah, B.W. Calcium-dependent protein kinase genes in corn roots. ASGSB Bulletin 10, 44, (1996).

Takezawa, D., Ramachandiran, S., Paranjape, V., and Poovaiah, B.W. Dual regulation of a chimeric plant serine/threonine kinase by calcium and calcium/calmodulin. J. Biol. Chem., 271, 8126-8132 (1996).

Wang, W., Takezawa, D., and Poovaiah, B.W. A potato cDNA encoding a homologue of mammalian multidrug resistant P-glycoprotein. Plant Mol. Biol., 31, 683-687 (1996).

Wang, W., Takezawa, D., Narasimhulu, S.B., Reddy, A.S.N., and Poovaiah, B.W. A novel kinesin-like protein with a calmodulin-binding domain. Plant Mol. Biol., 31, 87-100 (1996).

Mechanism of Auxin Action in Root Growth/Gravitropism

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Project Identification: 199-40-57-25
Initial Funding Date: 2/95
FY 1996 Funding: $116,993

Solicitation: 93-OLMSA-07
Expiration: 2/98
Students Funded Under Research: 6

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.

Last year, we reported that a plus/minus screening method failed to identify any auxin-regulated clones from seedling root libraries. This suggested that auxin may not alter the level of abundantly expressed messages in roots, and that more sensitive methods might be required to identify lower abundance up- and down-regulated messages. This year, we employed several PCR-based subtractive hybridization techniques to search for auxin regulated messages. One of these methods produced five gene fragments that appear to be auxin regulated. These gene fragments have been cloned, and at present, we are attempting to verify auxin regulation by RNase protection assays. We have also made good progress in characterizing H^+-ATPase transcripts in corn roots and have documented the alternative splicing of a novel H^+-ATPase pre-mRNA. One splicing pattern produces a

transcript, MHA 4, with autoinhibitory domain sequences. The alternative pattern results in a transcript, MHA 4d, lacking autoinhibitory domain sequences. We speculate that differential expression of MHA 4/MHA 4d messages could provide a novel and previously undiscovered mechanism to regulate the plasma membrane proton pump.

How plants respond to gravity to produce a predictable pattern of growth is an interesting problem in developmental biology and has important ramifications regarding our ability to grow and utilize plants in the microgravity environment of space. When a plant root is placed in a horizontal position, it begins to curve downward within minutes and reestablishes its original vertical orientation within several hours. This phenomenon, known as positive gravitropism, can be divided into three components: 1) gravity perception, 2) signal transduction, and 3) asymmetric cell elongation. Since the site of gravity perception is the root apex (likely the root cap), and asymmetric cell elongation occurs several millimeters distant in the zone of elongation, some signal(s) must migrate rapidly from the cap to the elongation zone. An important problem in plant gravitation research today is the nature of this signal and how it migrates to and influences events within the zone of elongation. There is a substantial body of evidence which suggests that auxin (IAA) is this signal. However, there are also serious questions and inconsistencies which cast doubt on auxin-based models. This controversy is unlikely to be resolved until we better understand the cellular and molecular events associated with the differential regulation of cell elongation in roots caused by IAA. This area of growth physiology has been neglected and understudied relative to the mechanism by which auxin promotes the growth of shoot cells. The experiments we are conducting represent steps which may help to rectify this situation and provide new tools to test whether IAA is indeed the gravitropic effector in roots.

Cellular Bases of Light-regulated Gravity Responses

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Project Identification: 199-40-57-47
Initial Funding Date: 2/96
FY 1996 Funding: $86,625

Solicitation: 95-OLMSA-01
Expiration: 2/99
Students Funded Under Research: 8

Task Description:

The overall objective of this research is to further define and inter-relate the cellular processes that are changed under the joint influence of gravity and light to produce gravitropic growth in plants. The proposed experiments are designed to test earlier inferences that Ca^{2+} plays a regulatory role in gravitropism by trying to identify one or more of the steps in the transduction chain for gravitropic growth in which Ca^{2+} is likely to exert a critical influence. Our recent studies on a Ca^{2+}-binding protein in pea seedlings called p35 indicate that it is a member of the annexin family of proteins, and, like animal annexins, may participate importantly in the regulation of Ca^{2+}-stimulated secretion. In particular our observation that p35 is most highly concentrated in cells that are secreting wall or other extracellular matrix materials have led us to propose that annexins may play a key role in growth regulation through its function in delivering materials needed for wall construction. The experiments proposed will test this hypothesis by determining whether p35 can, like animal annexins, promote vesicle fusion and ion transport changes, whether its expression is stimulated by light signals that promote orthogravitropic growth, and whether it accumulates in curved regions where asymmetric growth is induced by the gravitropic stimulus. Structural studies to better define the similarity of p35 to known annexins will also be performed. To further test whether Ca^{2+} is a signal transducer for light and gravitropic stimuli, we will study whether these stimuli induce a change in the level or distribution of cytoplasmic free $[Ca^{2+}]$ in fern rhizoid cells.

Here we summarize progress made during FY96 under three headings, related to the three aims stated in the proposal and approved for funding. All contribute to the overall goal of attaining a better understanding of the cellular functions that are altered by gravity and light to produce gravitropic growth. Aim 1 was to carry out studies on annexins that will reveal their complete primary structure and rationalize the production of specific antibody and oligonucleotide probes to distinguish possibly different roles for different plant annexins. Analysis of new sequences appearing in the data bank allowed us to deduce the apparent full-length sequence of an *Arabidopsis* annexin. Additionally, full-length sequences of two different annexins from *Zea mays* have been released to the data bases. Surprisingly, the results thus far suggest that unlike the case in most animal annexins the N-terminal region of plant annexins are uniformly short and relatively indistinguishable from one another. Although this result renders less likely the probability of developing specific antibody probes based on N-terminal sequence differences among the different annexins, the availability of full-length annexin sequences did allow us to short circuit our own project to obtain the full-length sequence of one or more pea annexins, and to proceed immediately to projects that would utilize the sequence information to learn more about the different functions of annexins in plants. These projects include (1) utilizing the yeast two-hybrid system to deduce the binding partner(s) of the *Arabidopsis* annexin, and (2) collaborating with Pioneer seed company to obtain mutant

seeds of corn in which mu transposons have inserted into annexin sequences, presumably disrupting these sequences and creating phenotypes resulting from defective or absent annexins. The two-hybrid system is at too early a stage to report any definitive results, but the Pioneer project has progressed nicely and we expect mutant seeds harboring errors in their annexin genes to be sent to us soon. Aim 2 of the project was to describe the expression and possible differential localization of annexins during gravitropic growth in various plant organs. Using polyclonal antibodies previously demonstrated to be specific to annexins in diverse plants ranging from ferns to corn, peas, and *Zinnia*, we have found that a gravitropic stimulus given to pea seedlings results in an enhanced expression of annexins near the growing tip of pea seedlings. Although this enhanced expression does not show a top-bottom asymmetry, our results do demonstrate a link between the gravitropic stimulus and the expression of a specific calcium-binding protein. Aim 3 was to further characterize the regeneration and polar development of protoplasts of prothallial cells of *Ceratopteris*. Two manuscripts related to this aim have been completed and will soon be submitted to *Planta* for review and possible publication. The first of these further characterizes the gravity-induced polarity of development in germinating spores of *Ceratopteris* and compares the relative strength of this response to that induced by light, and the second describes a method for purifying protoplasts from prothallial cells that will replicate the polarity of development seen in germinating spores, and it documents the effects of gravity and light on this polar development.

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.

FY96 Publications, Presentations, and Other Accomplishments:

Clark, G.B., Lloyd, A., and Roux, S.J. (abstract) Two-hybrid analysis using two different annexin genes from *Arabidopsis*. Plant Phys., 111 (supplement), 157 (1996).

Hsieh, H.-L., Tong, C.-G., Thomas, C., and Roux, S.J. Light-modulated abundance of an mRNA encoding a calmodulin-regulated, chromatin-associated NTPase in pea. Plant Mol. Biol., 30, 135-148 (1996).

Thomas, C., Mendenhall, J., and Roux, S.J. (abstract) Filament-forming properties of a major chromatin-bound NTPase from pea. Plant Phys., 111 (supplement), 129 (1996).

Re-Evaluation of the Role of Starch in Gravitropic Sensing

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Project Identification: 199-40-57-28
Initial Funding Date: 2/95
FY 1996 Funding: $84,978

Solicitation: 93-OLMSA-07
Expiration: 2/98
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 wild-type (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 one and two) 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).

In the last year Dr. Stan Vitha has focused on two projects that are well along, light grown *Nicotiana* mutants and starch overexpresser mutants (*Arabidopsis*). Those two projects are described below. In addition, he has obtained a fair amount of data trying to see the extent to which root phototropism interacts with gravitropism. Also, we have been trying to identify the wild-type gene corresponding the the *pgm* locus in *Arabidopsis*. This would be another way of answering whether there are two genes that affect gravitropism.

Two abstracts describing our progress have just been submitted to the Plant Physiology meetings:

1. Restoration by light of hypocotyl gravitropism in a starch-deficient mutant of *Nicotiana* correlates with amyloplast sedimentation. S. Vitha, M. Yang, F.D. Sack.

Dark-grown hypocotyls of a starch-deficient mutant (NS458) of *Nicotiana sylvestris* have severely reduced gravitropism (*Pl. Phys.* 94:1867). However, initial experiments indicated that gravitropism improved dramatically when NS458 seedlings were grown in light. The effects of light on mutant and wild-type (WT) gravitropic sensitivity and plastid sedimentation were analyzed quantitatively. Although NS458 hypocotyls were gravitropic, the threshold doses of reorientation (single or intermittent) required to elicit curvature were twice as long for NS458 as the WT. Since vertical growth rates were equal in both genotypes, this suggests that NS458 is about half as sensitive as the WT. Light microscopy and image analysis were used to estimate plastid volume in cells of endodermis, the cell layer that differentiates as a starch sheath in the WT. In both light and dark grown NS458, only 10% of the plastid volume is occupied by starch, whereas WT plastids are essentially filled with starch. Light-grown NS458 had plastids almost three times larger than in dark-grown NS458 plants, whereas in WT plants, light increased the volume only 50% compared to the dark. Plastids in light-grown NS458 were sedimented, presumably due to their larger size and greater total starch content compared to dark-grown NS458 hypocotyls which lack plastid sedimentation. Thus, the substantial restoration by light of gravitropism in NS458 correlates with the appearance of plastid sedimentation, a finding which supports the hypothesis that plastid mass and sedimentation function in gravitropic sensing. And the finding that light-grown NS458 hypocotyls are still not as sensitive as the WT supports the hypothesis that a full complement of starch is necessary for full sensitivity.

2. Gravitropism and tissue-specific phenotypes in plastid starch and sedimentation in the *Arabidopsis sex1* mutant. S. Vitha, M. Yang, T. Caspar, F. Sack

The *sex1* (starch excess) mutant of *Arabidopsis* (TC265) accumulates extra starch apparently through inactivation of a hexose transporter in the plastid envelope. The presumptive gravity sensing cells of the root and stem were examined to determine whether the mutation altered gravitropism, amyloplast sedimentation, and starch content. Stereological analysis of electron micrographs of the central cap cells did not reveal any differences between *sex1* and the WT in plastid size, plastid number and their proportion per cell, cell area, cell height and relative position of plastids in the cell. Plastids in the peripheral rootcap and in the body of the root had noticeably more starch in *sex1* compared to the WT. Both root growth and gravitropic sensitivity were comparable. In the stem endodermis, amyloplasts were about 70% larger and were sedimented over much more of the length of the stem in *sex1* compared to the WT. However, within the endodermis of a single plant, sedimenting amyloplasts from the apical region and non-sedimenting ones from the basal region did not differ in size. *sex1* but not WT seedlings contained sedimented amyloplasts at the base of the hypocotyl in the cortex as well as in the endodermis. These data indicate that the *sex1* mutation affects different tissues differentially and can induce "ectopic" sedimentation. However, amyloplast sedimentation is not simply a function of plastid size but is also regulated by cell-specific factors.

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, e.g., how do plants "know" which way is up? The long-term hypothesis that the mass of starch provides this signal requires further critical testing to establish its viability; testing is underway and is supported by the present grant. Knowledge of the basic mechanism of gravitropic sensing would be of wide biological interest, not just in the plant research community, but among all biologists and indeed with concerned citizenry including students interested in space tomatoes or in science fair projects.

FY96 Publications, Presentations, and Other Accomplishments:

Geisler, M., Yang, M., and Sack, F.D. (abstract) *TMM* differentially affects stomatal precursor cell formation and activity in *Arabidopsis* organs. *Arabidopsis* meeting at University of Wisconsin, June 1995.

Kern, V.D. and Sack, F.D. (abstract) Gravitropism and phototropism in *Ceratodon purpureus*. ASGSB Bull., 10, 97 (1996).

Nadeau, J.A., Geisler, M., and Sack, F.D. (abstract) Genetic dissection of stomatal development in *Arabidopsis*. FASEB Plant Developmental Biology Conference, Saxtons River, VT. 1996.

Sack, F.D. Plastids and gravitropic sensing. Planta, (in press).

Sack, F.D. and Schwuchow, J. (abstract) Protonemal gravitropism and amyloplast sedimentation in the mosses *Funaria* and *Physcomitrella*. ASGSB Bull., 9, 140 (1995).

Sack, F.D., Schwuchow, J., and Kern, V.D. (abstract) Gravitropism in high density media supports intracellular, statolith-based sensing in moss protonema (*Ceratodon*). ASGSB Bull., 10, 99 (1996).

Sack, F.D., Yang, M., and Geisler, M. (abstract) Mutational analysis of stomatal development in *Arabidopsis*. J. Cell. Biochem. Suppl., 21A, 440 (1995).

Wagner, T.A., Cove, D.J., and Sack, F.D. *wrong way response*, a positively gravitropic mutant in protonemata of the moss *Ceratodon*. Planta, (in press).

Wagner, T.A., Cove, D.J., and Sack, F.D. (abstract) A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon*. ASGSB Bull., 10, 98 (1996).

Wagner, T.A., Cove, D.J., Sack, F.D. "A positively gravitropic mutant mirrors the wild-type protonemal response in the moss *Ceratodon*" in "Plants in Space Biology." Edited by: Suge, H. Institute of Genetic Ecology, Tohoku University, Japan, pp 53-60, 1996.

Wagner, T.A., Schwuchow, J., Oakley, C.E., Oakley, B.R., and Sack, F.D. (abstract) Isolation and characterization of a γ-tubulin cDNA from the moss *Physcomitrella patens*. Pl. Physiol. Suppl., 108, 585 (1995).

Walker, L.M. and Sack, F.D. An ultrastructural analysis of the effects of reorientation on cell component distribution in gravitropic protonemata of the moss *Ceratodon*. Int. J. Pl. Sci., 158, (in press).

Yang, M. and Sack, F.D. The *too many mouths* and *four lips* mutations affect stomatal production in *Arabidopsis*. Pl. Cell, 7, 2227-39 (1995).

Yang, M. and Sack, F.D. (abstract) Analysis of phenotypes of stomatal cluster mutants in *Arabidopsis* cotyledons. *Arabidopsis* meeting at University of Wisconsin, June 1995.

Yang, M., Nadeau, J., and Sack, F.D. (abstract) Characterization of a *cytokinesis defective (cyd)* mutant in *Arabidopsis*. Am. Soc. Cell Biol., Abstract No. 1912, in Mol. Biol. Cell, Suppl., 7, 239a (1996).

Perception and Transduction of the Gravitational Stimulus

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Project Identification: 199-40-17-09
Initial Funding Date: 6/96
FY 1996 Funding: $98,575

Solicitation: 95-OLMSA-01
Expiration: 5/99
Students Funded Under Research: 6

Task Description:

The ability to respond appropriately to the environment is essential for plant life. Gravity is a ubiquitous environmental factor that provides a cue to plants that subsequently leads to the regulation of plant form and function. Gravity either induces a polarity in cells or uses an inherent polarity to further polarize receptor cells. The gravity-induced polarity of these cells then provides the biochemical and/or biophysical conditions that allow the subsequent morphological polarities like the differentiation of the root/shoot axis or gravitropism of plant parts to take place.

In this proposal we present experiments that are aimed at elucidating a mechanism of gravisensing used by statolith-free cells by continuing our investigations on the gravity-induced polarity of cytoplasmic streaming in characean internodal cells. In order to better understand the mechanism of gravisensing, we will use gel electrophoresis combined with Western blotting, affinity chromatography, and immunolocalization in order to determine the identity of the putative gravireceptor and its localization. We will also continue our work on determining how the vectorial energy inherent in the gravitational field is transduced into a biophysical signal that the cell uses in order to initiate the cellular activities that lead to the graviresponse. In order to accomplish this goal we will measure gravity-induced changes in the Ca^{2+} fluxes using Sr^{2+} as a tracer as well as changes in the intracellular free [Ca^{2+}] using Fura-2-tagged dextrans after artificially inducing the graviresponse using unidirectionally applied-hydrostatic pressure.

The fact that we are using single cells to study a graviresponse in plants means that we can exploit the cell biological tools and principles that have been developed to study stimulus perception and signal transduction. However we believe that our conclusions can be generalized to explain gravisensing in higher plants and animals (i.e., the sense of balance).

This year we have continued to work on how a gravitational stimulus influences the flux of Ca^{2+} across the plasma membrane by following the flux of Sr^{2+} and quantifying the intracellular [Sr] with inductively coupled plasma emission spectroscopy. We found that in vertical cells, the flux of Sr^{2+} increases from 20 to 60 nmol/m2.s at the end of the cell that experiences tension, while the flux remains unchanged or decreases insignificantly at the end that experiences compression. The gravity-induced increase in the flux of Sr^{2+} into the top of the cell is inhibited by nifedipine, verapamil, and ω conotoxin. Moreover, RGDS inhibits the

gravity-induced increase in the Sr^{2+} flux, but only when it is applied to the site that experiences a gravity-induced tension. By contrast, YIGSR, an oligopeptide that inhibits gravity sensing at the site of compression, has no effect on the Sr^{2+} flux. Thus the cell experiences two distinct responses to gravity, one at the site of tension which influences the flux of Ca^{2+}, and one at the site of compression that has no influece on the flux of Ca^{2+}.

In order to see whether or not microtubules may be involved in gravity sensing, we treated the cells with amiprophosmethyl (APM) and found that APM causes a reduction of the polar ratio in a dose-dependent manner. We also find that APM completely inhibits the gravity-induced increase in Sr^{2+} flux into the top of the cell, even when we treat the bottom of the cell with APM. These results indicate that microtubules are involved in gravity sensing and in coupling the gravitational stimulus to the activation of Ca^{2+} channels at the top of the cell. Because APM is able to inhibit the gravity-induced Sr^{2+} flux at the top of the cell even when it is applied to the bottom of the cell, microtubules may be the structure that is involved in integrating the tension the cell experiences at the top with the compression the cell experiences at the bottom and converting these mechanical signals into a signal that activates Ca^{2+} channels and initiates the observable graviresponse.

We are continuing to test whether our model is applicable to higher plants and whether or not the statolith-actin microfilament model is robust enough to withstand critical testing. Other researchers have shown that cytochalasin D affects the polarity of columella cells, the velocity of statolith sedimentation and gravity-related changes in membrane potential. We have developed a procedure to visualize the microfilaments in columella cells with a light microscope and found that even though cytochalasin D disrupts the actin microfilaments in the columella cells of roots, it has no effect on gravitropism, thus calling into question the stotolith-actin microfilament model. We have also found that the specific rate of gravitropism, but not the rate of statolith sedimentation is inhibited by increasing the density of the external medium. These data can be explained by the gravitational pressure model, but not the statolith-actin microfilament model of gravitropism.

Our work has contributed to understanding the importance of the extracellular matrix in influencing physiology and development. It has also contributed to understanding the importance of calcium in signal transduction pathways and in the development of polarity. In terms of medicine, understanding the mechanism of gravisensing at the cellular level may contribute to understanding and treating the problem of loss of balance that is experienced by elderly people.

FY96 Publications, Presentations, and Other Accomplishments:

Staves, M.P., Wayne, R., and Leopold, A.C. Cytochalasin D does not inhibit gravitropism in roots. Am. J. Bot., (in press).

Staves, M.P., Wayne, R., and Leopold, A.C. The effect of the external medium on gravitropic curvature of rice (*Oryza sativa*) roots. Am. J. Bot., (in press).

Staves, M.P., Wayne, R., and Leopold, A.C. The effect of the external medium on the gravity-induced polarity of cytoplasmic streaming in *Chara*. Am. J. Bot., (in press).

Wayne, R. and Staves, M.P. A down to Earth model of gravisensing. ASGSB Bull., (in press).

Wayne, R. and Staves, M.P. A down to Earth model of gravisensing or Newton's Law of Gravitation from the apple's perspective. Physiol. Plant, 98, 917-921 (1996).

Wayne, R. and Staves, M.P. Connecting undergraduate plant cell biology students with the scientists about whom they learn: A bibliography. Am. Biol. Teacher, (in press).

Wayne, R. and Staves, M.P. The August Krogh Principle applies to plants. BioSci., 46, 365-369 (1996).

Remote Sensing for Research and Control of Malaria in Belize

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Funding:

Project Identification: 199-55-27-03
Initial Funding Date: 2/95
FY 1996 Funding: $366,089
Joint Agency Participation: DoD (USUHS)

Solicitation:
Expiration: 1/98
Students Funded Under Research: 7

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.

Geographic information system and remote sensing capabilities were established within the malaria control program, Ministry of Health, Belize. All village nodes were registered within the GIS and malaria case data were attached to each village. Landsat data formed a functional component of the GIS. Dry season predictions were tested against wet season conditions. The distance from 41 village sites to the nearest *An. albimanus* habitat

(macrophyte marsh) was used to predict high, medium, and low *An. albimanus* abundance in the villages. The predictions were nearly identical to earlier predictions developed for 13 villages included in a dry season study. In preliminary analyses there was a general pattern of villages closest to habitats presenting higher mosquito densities. In June 1996 an Investigator's Working Group (IWG) meeting was held in Belize. Research plans and timelines were developed for all project participants and their respective activities. After this workshop, a comparison of the actual annual parasite indexes versus actual measures of distance of villages from habitats revealed a negative relationship in numbers of cases of malaria with distance from the remotely-sensed habitats, which was a preliminary confirmation of the vector studies. Detailed malaria data by season and by age-sex distributions of cases in each village have been compiled and are now being analyzed for final correlation with earlier predictions. To fully characterize the role of different vector species as malaria vectors in central Belize, a survey program of 20 villages was initiated in August 1996. Ten villages were highly malarious and 10 villages were characterized with low levels of malaria. These data will be used for prioritizing vector species for future prediction studies and for defining the environmental conditions of malaria risk. Risk factors will be studied with remote sensing data. Images from 1996 will be used in future analyses and predictions. Major emphasis is presently being placed on the continued development of GIS and remote sensing analysis capabilities in Belize and the application of these capabilities to the malaria control effort.

Malaria is the preeminent reemerging disease in the Americas. The application of remote sensing technology to the study of this disease and its mosquito vectors is providing new and critical information for the proper management of malaria in developing countries. This research definitely seeks to understand the dynamics of a human disease on Earth. The research goal is to test the applicability of predictive models based on the use of multispectral satellite data to target applications of malaria control measures. Successful, cost-effective applications of remote sensing technology to the Belize National Malaria Control Program will have broad implications for malaria control throughout the World. This program of research has already resulted in a critical revision of our understanding of malaria epidemiology in Belize. As background, when we initiated research, the only recognized vector of malaria in Belize was *Anopheles albimanus*. Historically, all surveys and studies focused entirely on this vector species. However, our broad-based program of research has shown that at least four species are potentially important vectors of human malaria. We can now characterize these vectors by specific environments and seasons and that environmental surrogates can be employed, in combination with remote sensing data, to predict the time and location of malaria risk conditions. Thus, we are showing that satellite data can be used to predict where and when humans are at risk of malaria 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. The careful targeting of houses for insecticide spraying will not only reduce the total amount of malaria within the human population, it will also reduce the total amount of insecticide used for malaria control.

FY96 Publications, Presentations, and Other Accomplishments:

Manguin, S., Roberts, D.R., Andre, R.G., Rejmankova, E., and Hakre, S. Characterization of *Anopheles darlingi* (*Diptera: Culicidae*) larval habitats in Belize, Central America. J. Med. Entomol., 33(2), 205-211 (1996).

Rejmankova, E., Roberts, D.R., Manguin, S., Pope, K.O., Komarek, J., and Post, R. *Anopheles albimanus* (*Diptera: Culicidae*) and *Cyanobacteria*: An example of larval habitat selection. Pop. Ecol., 25(5), 1058-1067 (1996).

Rejmankova, E., Roberts, D.R., Pawley, A., Manguin, S., and Polanco, J. Predictions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. Am. J. Trop. Med. Hyg., 53(5), 482-488 (1995).

Roberts, D.R., Paris, J.F., Manguin, S., Harbach, R.E., Woodruff, R., Rejmankova, E., Polanco, J., Wullschleger, B., and Legters, L. Predictions of malaria vector distribution in Belize based on multispectral satellite data. Am. J. Trop. Med. Hyg., 54(3), 304-308 (1996).

Roberts, D.R., Rejmankova, E., Pawley, A., Paris, J., Manguin, S., Polanco, J., and Legters, L. Remote sensing as a tool for predicting high risk areas for malaria transmission in Belize. 46th International Astronautical Congress. Oslo, Norway. IAF-95-B.5.10. 7pp (October 2-6, 1995).

NSCORT: Integrated Physiology

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Funding:

Project Identification: 199-93-17-08
Solicitation:
Initial Funding Date: 6/93
Expiration: 5/98
FY 1996 Funding: $1,126,000
Students Funded Under Research: 24

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 control of myocardial contractile performance. Section II on mineral metabolism explores the mechanisms that are involved in bone loss and hypercalcuria as induced by immobilization or exposure to microgravity and its prevention. Section III on skeletal muscle structure and function has three components studying (a) human inborn defect of oxidative metabolisms, (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 bedrest. Project (a) examines the role of cardiac mechanics in orthostatic intolerance; project (b) examines the regulation of peripheral blood flow, including cerebral perfusion, in deconditioned human subjects; and project (c) studies 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 to support of formal graduate school education to post-doctoral research fellowships. The NSCORT and its investigators have had an important role in the development of a new graduate program (Ph.D.) in Integrative Biology within the core unit of the graduate school at UT Southwestern, the Division of Cell and Molecular Biology.

June 1, 1997 is the fourth anniversary of the NSCORT in Integrated Physiology. Multiple and strong links have been developed between the units of the center. The new Ph.D. program has unique features and represents an important new development.

Significant progress has been made in several research areas, including definition of important cellular and molecular mechanisms that control atrophy and hypertrophy of skeletal muscle (DeMartino) and myocardial performance (Radford). This work has included studies of intracellular protein degradation in skeletal muscle and of the metabolic links between mitochondrial function and cardiac performance. The unit on mineral metabolism (Pak) has made significant progress developing pharmacological treatment against bone loss. The three units in the section on skeletal muscle structure and function have (a) develped new technologies using C^{13} analogues to define pathways of substrate utilization, (b) used patients with metabolic myopathies as models of disuse atrophy (Haller), and (c) explored MRI imaging to examine muscle fluid shifts and injuries (Fleckenstein). The section on human cardiovascular physiology has provided important new information on the effect of simulated microgravity on the central and peripheral circulation (Levine). Prolonged bedrest significantly affects the mechanical properties of the human myocardium and the loss of cardiac muscle mass is associated with increased stiffness. Studies of the interactions between arterial and cardiopulmonary baroreflexes (Raven), the control of the cutaneous circulation (Crandall), and of the interactions between central and peripheral nitric oxide systems and sympathetic outflow (Victor) have also provided new information.

Members of the NSCORT are also actively involved in space flight experiments scheduled for Neurolab and Mir.

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 bedrest). Our NSCORT unit on mineral metabolism has developed new and effective methods to prevent mineral loss and stone formation in the urinary tract, methods applicable both to space and general medicine. Studies of cardiovascular dysfunction following actual and simulated microgravity have provided new insights into the mechanisms involved in orthostatic hypotension, an important condition that is commonly encountered in general medical practice. 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.

New findings include information on the effects of simulated microgravity (bedrest) on the mechanical properties of the heart and blood vessels and on the control of blood flow to the brain.

FY96 Publications, Presentations, and Other Accomplishments:

Bertocci, et al. 13C fractional enrichments can be measured in rat skeletal by 1H MRS. J. Appl. Physiol., (in press).

Bertocci, et al. 13Exogenous C-labeled substrates can be administered to rat skeletal muscle and taken up sufficiently for subsequent 13C isotopomer analysis. J. Appl. Physiol., (in press).

Buckey, J.C., Gaffney, F.A., Lane, L.D., Levine, B.D., Watenpaugh, D.E., Wright, S.J., Yancy, C.W., Jr., and Blomqvist, C.G. Central venous pressure in space. J. Appl. Physiol., 81(1), 19-25 (1996).

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 following spaceflight. J. Appl. Physiol., 81(1), 7-18 (1996).

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., 81(2), 686-694 (1996).

Levine, B.D., Zuckerman, J.H., and Pawelczyk, J.A. Cardiac atrophy after bedrest deconditioning: A non-neural mechanism for orthostatic intolerance. Circulation, (in press).

Shi, X., Gallagher, K.M., Smith, S.A., Bryant, K.H., and Raven, P.B. Diminished forearm vasomotor response to central hypervolemic loading in aerobically fit individuals. Med. Sci. Sports Exerc., 28, 1388-1395 (1996).

Vissing, J., Galbo, H., and Haller, R.G. Exercise fuel mobilization in mitochonrial myopathy: A metabolic dilemma. Ann. Neurol., 40, 655-662 (1996).

Vissing, J., Galbo, H., and Haller, R.G. Paradoxically enhanced glucose production during exercise in humans with blocked glycolysis due to muscle phosphofructokinase deficiency. Neurology, 47, 766-771 (1996).

NSCORT: Radiation Health

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Funding:

Project Identification: 199-93-17-07
Initial Funding Date: 1/92
FY 1996 Funding: $941,000

Solicitation: NRA
Expiration: 12/96
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.

Progress was made in theoretical and experimental studies of various biological systems irradiated with HZE particles. These studies are of direct relevance with respect to NASA's long duration space flights. The theoretical studies use Monte Carlo techniques to calculate DNA strand break production by direct and indirect effects of ionizing radiation and are based on general features of track structure, stopping power theory, and incorporate detailed coordinate models of chromatin fibers of DNA. The chromatin fibers are assumed to be immersed in liquid water containing a generalized OH radical scavenger such that the characteristic migration distance of OH radicals is approximately 4 nanometers as observed in mammalian cells. Tracks are generated at random with respect to the chromatin fiber, and damage to sugar and base sites results from interactions with radiolytic products of water and from direct excitation/ionization.

We have incorporated into our calculations both solenoidal and ribbon models (in two different forms) of the 30 nm chromatin fiber. The conventional solenoid model, which we have described in detail in recent publications, consists of an idealized super-helix with six nucleosomes per turn. Recent electron microscopic tomography results suggest that chromatin fibers may, instead, be organized as a continuously variable zig-zag nucleosomal ribbon. We have, therefore, developed detailed coordinate models of ribbon fibers by successively joining nucleosomes and linkers.

Theoretical studies have demonstrated the formation of small fragments, intermediate fragments, and large fragments of DNA along with clusters of damages involving several sugar and base damages.

We introduced a new methodology of particular importance for examining the repair processes further. DSBs in selected regions of the genome can be directly quantitated by hybridization detection of unique large (e.g. NotI) restriction fragments separated by PFGE, thus providing the potential to address intra-genomic heterogeneity in either induction or repair of damage. Moreover, when used to follow repair, the technique measures rejoining of correct DNA ends by the criterion of reconstitution of the hybridizing NotI restriction fragment; when taken together with measures of total rejoining, it is thus possible to estimate the misrejoining frequency. Using this approach, we measured the misrejoining frequency of DNA double-strand breaks induced by X-rays (Löbrich *et al.* 1994, 1995) and by high LET particles including high-energy Fe ions from the AGS (preliminary results shown below). We found that a surprisingly large proportion (25%) of the breaks were misrejoined regardless of the LET of the particle. This study was performed with non-cycling cells at relatively high doses, which makes direct comparison with the formation of chromosomal aberrations inconclusive.

For mutational studies, we have shown that low fluence exposures to high energy Fe ions produce predominantly deletion mutations at the tk and hprt loci in TK6 cells and along chromosome 11 in S1-deficient mutants in the human x hamster hybrid cell line, AL. Protons also produce deletions, but the number of particle traversals to produce similar levels of mutations is roughly two orders of magnitude greater than is required for Fe-induced mutations. Using three mutation target loci in the two different cell models, we demonstrated that the position of the target locus relative to essential genes strongly influences the recovery of viable mutations following exposures to Fe ions, protons, and helium ions in addition to photons.

These data from microenvironmental studies suggest that radiation-induced cell kill is more complicated than previously believed and that there are alternatives to DNA damage as the critical event. Likewise the concepts of how carcinogenesis is initiated and promoted have also evolved. While mutations are solidly implicated in all stages of the process, it is clear from many studies that any single mutation is not sufficient, that the critical mutations vary as a function of the cell and tissue of origin, and that genomic instability may be the only feature common to all cancers. Further, quantitation of the initiation step suggests that it is far more frequent than progression which suggests that mechanisms controlling the frequency of tumor progression may be more important to controlling the outcome of carcinogen exposure.

Data from our studies with gamma, proton, and HZE particles (detailed in preliminary results) illustrate the potential of radiation-induced extra-cellular signaling to orchestrate global changes in tissue composition, demonstrate that critical signaling pathways can be manipulated *in vivo*, and suggest that the radiation response of tissues is mediated by extra-cellular mechanisms that can coordinate the response of multiple cell types,

presumably directed towards tissue recovery. However, this response may be deleterious under chronic conditions, such as continuous low fluence exposure encountered in space, or may be compounded by other physiological responses, such as microgravity or stress.

Ionizing radiation plays a very important role in our everyday life. The technological and medical applications of radiation and radioactivity have a long history. In addition to these benefits, ionizing radiation can be hazardous to humans, both on ground and in space. Hence, radiation can be beneficial as well as risky. It is extremely important that we understand at a fundamental level, the effects of ionizing radiation on living cells, tissues, and organs. Research in this project addresses many questions related to these understandings through basic research. Much of the investigation is focused towards human cancer-induction as well as cure of this disease. As far as induction of cancer is concerned, the findings of the research are equally applicable on Earth and in space.

In addition, this research also addresses radiation-induced cataractogenesis. The results of our study, which quantitatively 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.

FY96 Publications, Presentations, and Other Accomplishments:

Ehrhart, E.J., Gillette, E.L., and Barcellos-Hoff, M.H. Immunohistochemical evidence of rapid extracellular matrix remodeling after iron-particle irradiation of mouse mammary gland. Radiat. Res., 145, 157-162 (1996).

Ehrhart, E.J., Gillette, E.L., and Barcellos-Hoff, M.H. (abstract) Radiation quality comparison of TGF-ß activation in mouse mammary gland. Proceedings of the Xth International Congress of Radiation Research, Wurzburg, Germany.

Holley, W.R. and Chatterjee, A. Clusters of DNA damage induced by ionizing radiation: Formation of short DNA fragments. I. Theoretical Modeling. Radiat. Res., 145, 188-199 (1996).

Löbrich M., Rydberg B., and Cooper P.K. Assays for determining double-strand break induction and rejoining quality in specific genomic locations. Radiation Research 1895-1995, Proceedings of the Xth International Congress of Radiation Research (in press).

Rydberg, B. Clusters of DNA damage induced by ionizing radiation: Formation of short DNA fragments. II. Experimental Detection. Radiat. Res., 145, 200-209 (1996).

NSCORT: Environmental Health

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Funding:

Project Identification: 199-93-17-02
Initial Funding Date: 1/91
FY 1996 Funding: $1,044,000

Solicitation: 94-OLMSA-04
Expiration: 12/95
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 information to support models that approximate human response to contaminants and conditions in space habitats. The degree to which these models deviate from actual conditions in space will contribute to our understanding of the role of gravity, confinement, and radiation. Such models will make visible the pervasive but invisible role of space constraints like gravity and confinement in the human response to stress from contaminants. Indeed, at the most basic scientific level, the distinguishing feature of space environmental health is the study of the role of gravity and confinement in determining human health risks from chemicals, airborne particles, microorganisms, and viruses. Physical phenomena that depend on the force of gravity, weight, density, convection, sedimentation, and hydrostatic pressure definitely play a role in the vestibular, musculoskeletal, and endocrine systems and may play a role in human risk from environmental contaminants. Thus, airborne particles, in the absence of sedimentation and convective flow, persist for longer periods in the atmosphere. The human host, compromised by microgravity-related effects—reduced red cell mass, calcium loss, muscle atrophy, diminished immune response—may respond differently to toxic or infective stress than in normal gravity. Confinement may also play a critical role in these processes and may affect human neuroresponses.

The specific goal of this Center is to conduct ground-based research to minimize health risks so that the survival and productivity of astronauts are not compromised by contaminants or other environments in the spacecraft, and to train investigators in life sciences, medicine, engineering, and the physical sciences in this new subdiscipline of space environmental health. This Center will focus on two major sources of health risks: airborne chemicals and particulates, and recycled water contaminants. In addition, the Center promotes generic projects for the assessment of risk and the development of modeling tools to assess the environmental health state of the habitat and crew during long-term space flight.

The research conducted by this NSCORT is carried out by teams of biological scientists and engineers. This report, therefore, will summarize team accomplishments rather than individual work.

The Inhalation Risk Team. The major discovery by this team is that ultrafine particles, liberated by the overheating of polytetrafluoroethylene (teflon), are highly toxic to the lung. This year's experiments clearly implicated ultrafine particles as opposed to other substance released from teflon. The overheating of teflon, such as might occur with electrical insulation, produces large numbers of ultrafine particles and gaseous materials.

Studies on rats revealed that only the ultrafine particle component was able to induce inflammation. The inflammatory response is mediated by cytokines generated by lung macrophages. A second series of experiments revealed that brief exposures to ultrafine particles can protect against longer exposures that otherwise would induce inflammation. The brief pre-exposures up-regulated antioxidant and anti-inflammatory defense mechanisms.

The Human Performance Risk Team was established in this NSCORT because any deleterious effects on astronaut performance could critically affect manned space missions. Toluene is present in spacecraft due to degassing from plastics and other polymeric materials. Generally, toluene is found at concentrations higher than other volatile organic solvents. Studies were conducted on six healthy volunteers who were exposed, in 6 hour periods, to toluene at the industrial threshold limit value of 100 ppm, an air concentration hitherto believed to be non-toxic. No effects were found on lung function but certain aspects of performance were adversely affected. As determined by a one hour complex performance test (SYNWORK), the composite score was about 10% lower in the last hour of exposures to toluene as compared to controls. Differences in performance between air alone and toluene were greatest after exercise. Unfortunately due to cessation of funding, the team has not been able to test for effects of additional stresses such as sleep deprivation and infection, stresses that may well be important in space missions.

The Water Recycle Team, at the start of this NSCORT, constructed a water recycle test bed to study the growth and persistence of viruses in recycled water and to test the effectiveness of disinfection techniques. MS-2 strain coliphage was chosen as the model virus and iodine as the disinfectant. This year analytical methods have been refined to identify and measure iodine disinfection products. The modified method allows the measurement of iodoform which is likely to be the principal disinfection product. The formation of biofilms has been found to play a key role in the persistence of viruses and resistance to disinfection. Studies have been conducted on the role of molecular size and charge on biofilm of organic matter and on the effect of ozonation of the sorption of natural organic matter by biofilm.

The Quantitative Risk Assessment Team is to quantifying health risks to astronauts based on the research findings of this NSCORT. Thus, as the NSCORT teams have developed new data, the role of the Risk Assessment Team has grown in importance. This year a seven-step model was completed for the selection and general placement of sensing devices for airborne contaminants in a predefined space habitat. The latter was assumed to take the form of three interconnected space station modules. The contaminants were chosen to be hydrazine, ammonia, and carbon monoxide. The full model was reported in a student thesis completed in December 1996. Specifically, utilizing the designed contaminant monitoring system reduced the simulated costs associated with severe contamination events by 50%.

The Training and Outreach program has seen the graduation of two masters and four Ph.D. students who have now taken positions in academia or in space-related industries. Four undergraduate minority students were given hands-on laboratory experienced in the summer months of 1996. The outreach program has been responsible for presentations in high schools, science museums, and to civic audiences such as retired professionals and veterans. In fact, the outreach program is receiving increasing requests for educational presentations and displays, a somewhat embarrassing situation in view of the abrupt discontinuation of this NSCORT.

The research tasks are directly relevant to understanding certain human disease processes. The study on ultrafine particles has led to the hypothesis that such particles may contribute to human morbidity on earth. Indeed, we now suspect that lung function in areas of air pollution such as in the large industrialized cities may in part be due to the inhalation of ultrafine particles. Such particles are not normally detected by the commonly used filters for airborne particulate pollutants. Thus, this project has given rise to a new approach to assessing the causes of lung damage from air pollution.

The studies on toluene have also given new insights into the Earth-based problem of indoor air pollution both in the workplace and in the home. People are increasingly finding themselves having to perform complex tasks in situations with multiple stresses. This study has already alerted the occupational medicine community that

subtle effects of chemicals on complex performance tasks can and do occur at air levels of toluene hitherto believed to be safe. Future studies would have examined the combined effects of several stresses such as sleep deprivation, cold or flu infections, and exposure to airborne pollutants to mimic real life workplace conditions in an increasingly sophisticated work environment.

The persistence of viruses in drinking water remains a major public health concern especially in third world countries. Infant mortality can still reach appalling levels, even exceeding 50% in countries with poor sanitation. Our studies in water disinfectants and the formation of bioflims that hinder the disinfection process are directly related to these ground-based public health problems.

FY96 Publications, Presentations, and Other Accomplishments:

Frazey, P.A., Barkley, R.M., and Sievers, R.E. Analysis of iodocarbon compounds in water and air by solid phase microextraction. American Chemical Society, Rocky Mountain Regional Meeting, Denver, CO (June 1996).

Frazey, P.A., Helmig, D., and Sievers, R.E. Selected ion monitoring - Mass spectrometric analysis of iodocarbons inambient air. Atmospheric Chemistry Symposium, Boulder, CO (November 1996).

Irons, R.D., Coagiovanni, D.B., and Stillman, W.S. Murine thymic lymphoma is associated with a species-specific hematopoietic progenitor cell subpopulation. Toxicology, 113(3), 59-57 (1996).

Johnson, C.J., Finkelstein, J.N., Gelein, R., Baggs, R., and Oberdoerster, G. Characterization of the early pulmonary inflammatory response associated with ultrafine PTFE-particle induced exposure. Toxicol. Appli. Pharmacol., 140, 154-163 (1996).

Oberdörster, G. "Effects of ultrafine particles in the lung and potential relevance to environmental particles" in "Aerosol Inhalation: Recent Research Frontiers." Edited by: Marijnissen, J.C.M. and Gradon, L. Kluwer Acacademic Publications, The Netherlands, pp 165-173, (1996).

Oberdörster, G. Significance of particle parameters in the evaluation of exposure dose-response relationships of inhaled particles. Inhal. Toxicol., 8, 73-90 (1996).

Rahill, A., Morrow, P.E., Frampton, M.W., Cox, C., Gibb, R., Gelein, R., Speers, D., and Utell, M.J. Human performance during exposure to toluene. Aviation, Space and Environ. Med., 67, 640-647 (1996).

Smith, G. and Morgenthaler, G. Space habitat environmental health risk assessment and management. Space'96. The 5th International Conference and Exposition on Engineering, Construction, and Operations in Space, Albuquerque, NM (June 1-6, 1996).

Weiss, B. and Elsner, J. The interaction of risk assessment and neurobehavioral toxicology. Env. Health Perspect., 104 Suppl. 2, 173-177 (1996).

NSCORT. Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology

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Funding:

Project Identification: 199-93-17-14
Initial Funding Date: 1/96
FY 1996 Funding: $999,634

Solicitation: 94-OLMSA-04
Expiration: 12/00
Students Funded Under Research: 69

Task Description:

This program in gravitational biology involves 9 faculty members from 2 colleges at NCSU, 1 from Wake Forest University, 1 from Baruch College, and 1 from Kennedy Space Center. The overall goal is to study calcium as a central focal point in the gravity response. The group uses an integrated molecular, cellular, and physiological approach to plant gravitational biology.

The precise modulation of calcium homeostasis will be acheived using transgenic technologies and monitored using sophisticated imaging techniques to verify the specificity and extent of transgenic expression. These efforts, in combination with our expertise in local and long-distance signalling, will make a major contribution to understanding the fundamental role of calcium in orchestrating the transduction of the gravity stimulus into an autopoietc (self-regulated) response.

The project brings together experts in a range of specially-selected fields to address a single major research problem, i.e., the fundamental role of calcium in regulating gravity-stimulated signal transduction in plants. The expertise to be called on includes molecular biologists to produce transgenic plants with altered calcium homeostasis (Thompson, Robertson, Sederoff); cell biologists to image calcium and other components of the signal transduction pathway (Allen, Tucker); physiologists to study signal transduction (Boss, Davies, Muday); and biochemists (Brown, Huber, Huber) to study calcium-modulated carbon/nitrogen metabolism. By fostering interdisciplinary collaborations among these diverse laboratories, the proposed program will create a multi-faceted approach to the problem of plant gravitational biology.

The NSCORT is a consortium of institutions including North Carolina State University (College of Agriculture and Life Sciences and School of Forestry), Wake Forest University, Baruch College (City University of New York), NASA's Kennedy Space Center and Dynamac Corporation (which runs the Life Science Support contract at KSC). Faculty, staff and/or students from all of the institutions participate in various aspects of the program. It consists of three major components: Education, Outreach and Research.

Considerable progress has been made in all three areas. For the Education component, a new graduate level course in Gravitational
and Space Biology was initiated and taught under the leadership of Christopher Brown. This class was offered simultaneously at six university campuses across the state through a statewide microwave network. For the Outreach component, a summer workshop for high school teachers entitled "Plants and Gravity" was developed and implemented under the leadership of Joan Huber. Nine teachers from two states spent time at the NCSU campus interacting with project leaders and research associates in the program. Already some of the teachers have utilized the experience to develop new curricula at their schools. We plan to build and expand on these accomplishments throughout the next year in order to reach the maximal number of students and educators with the highest quality science education and science education ideas.

For the Research component, under the leadership of Wendy Boss, these first few months were taken up with hiring postdoctoral research associates and renovating facilities. We have revised our original organizational chart involving six research sections and reduced them to three: altering calcium homeostasis; signal perception and transduction; and gravity responses. Our first tasks were to begin developing the biological systems most appropriate for our integrated, combinatorial approach, and to select targets for genetic manipulation of calcium homeostasis. With the establishment of a cadre of postdoctoral fellows has come a synergism that has stimulated exciting collaborations that are reflected in our 1997 goals.

This research will determine the mechanisms by which plants, including the model plant, *Arabidopsis*, perceive and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions, will improve our understanding of how they grow in various space conditions (Earth orbit, Mars, etc.) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products of enhanced paper quality (pine xylem system and its formation of compression wood) and yield of seed grains (reorientation of corn plants blown over in strong winds).

Work on a novel form of a Kelvin Bio-probe, being done in conjuction with the Wood's Hole Marine Biology Lab, will aid in the development of new techniques to measure growth and surface potential of 3-dimensional tissues on a real time basis. This technique will then be applicable to studies on plants growing in a variety of Earth and space conditions in order to elucidate the response of plants to their surrounding environment.

NSCORT: NASA/NSF Joint Program in Plant Biology

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No Co-Is Assigned to this Task

Funding:

Project Identification: 199-93-17-11

Initial Funding Date: 9/94

FY 1996 Funding: $515,000

Joint Agency Participation: National Science Foundation

Solicitation:

Expiration: 8/99

Students Funded Under Research: 38

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.

As described in the FY95 report, one of the key developments at the 1996 meeting was a decision to hold a special meeting in order to define a "Super Project" that could serve as a focal point for the coordination of network efforts in studying plant sensory systems. This special meeting was held in Half Moon Bay, CA on February 28 - March 1, 1996. The major outcome of the meeting was a group decision to focus on "A Physiological and Molecular Characterization of the Distal Elongation Zone in Roots" as a central project on plant sensory perception (see report from Half Moon Bay meeting submitted March 1996). In addition to this meeting, we held the second annual meeting of the NASA/NSF Network for Research on Plant Sensory Systems at Washington University in Saint Louis on October 2-4,1996. Although this meeting took place only 7 months after the Super Project meeting in California, the focus of the Saint Louis meeting was on initial progress in the Super Project.

Our primary goals for the first year of the super project were to isolate sufficient quantities of root tissue subsections from *Arabidopsis* to allow sufficient extraction of mRNAs to do differential display analysis of gene activation in the tissue subregions and to prepare to utilize the gene micro array technology of the Davis laboratory to determine genes uniquely expressed in the DEZ or uniquely activated by gravistimulation. A second major goal was to begin characterizing the electrophysiological properties of cells from the DEZ. We have met these goals. The Evans lab has developed a method for isolating large numbers of tissue specific regions from the tiny roots of *Arabidopsis* and tissue collections have been forwarded to the Kieber lab. The Kieber lab has extracted mRNA and is running the differential displays for analysis. Meanwhile the Spalding laboratory has developed a method for isolating and patch clamping protoplasts from the DEZ and is making progress in characterizing the ion channels present in these cells.

In addition to this work, collaborative efforts begun during the first year of the operation of the network have continued (see publications) and new collaborative efforts have begun with emphasis on the super 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.

In additon to these benefits, there are two general benefits to the research community to be realized from network activity. One derives from our progress in cloning genes from each developmental region of the *Arabidopsis* root. Once cloned these genes will be useful tools for the research community at large in a wide variety of investigations of plant development and sensing. A second benefit derives from the networks ongoing development of a plant growth imaging web site devoted to automated analysis of plant growth and standardization of plant growth experimental conditions. This is anticipated to provide a means for greatly accelerating our progress in the understanding of plant growth.

FY96 Publications, Presentations, and Other Accomplishments:

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Ishikawa, H. and Evans, M.L. Specialized zones of development in roots. Plant Physiol., 109, 725-727 (1995).

Ishikawa, H. and Evans, M.L. Novel software for analysis of root gravitropism: comparative response patterns of *Arabidopsis* wild type and *axr1* seedlings. Plant Cell & Environ., (in press).

Kieber, J.J. The ethylene signal transduction pathway in *Arabidopsis*. J. Expt. Bot., 48 (in press).

Lewis, B.D. and Spalding, E.P. (abstract) An important role for Ca^{2+} in the activation of anion channels by cold but not blue light. Annual meeting, American Society of Plant Physiologists, San Antonio. Plant Physiol. 111: Suppl. 107 (July 1996).

Pallin, J.B., Woeste, K.E., and Kieber, J.J. "Role of the CTR1 kinase in ethylene signal transduction in *Arabidopsis*" in "Protein Phosphorylation in Plants." Edited by: Shewry, P. R., Halford, N. G., and Hooley, R. Proc. Phyto. Soc. Europe. Clarendon Press: Oxford, pp 255-265, (1996).

Parks, B.M., Cho, M.H., and Spalding, E.P. (abstract) Rapid electrical and growth responses induced by blue light in *hy4* and wild-type *Arabidopsis* seedlings. Annual meeting, American Society of Plant Physiologists, San Antonio, Plant Physiol 111: Suppl 154 (July 1996).

Pei, Z.-H., Ward, J.M., Harper, J.F., and Schroeder, J.I. A novel chloride channel in *Vicia faba* guard cell vacuoles activated by CDPK, a calcium dependent protein kinase with a calmodulin-like domain. EMBO, 15, 6564-6574 (1996).

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NSCORT: Bioregnerative Life Support

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Project Identification: 199-93-17-12
Solicitation: 94-OLMSA-04
Initial Funding Date: 1/96
Expiration: 12/00
FY 1996 Funding: $ 1,000,000
Students Funded Under Research:

Task Description:

Research at NJ-NSCORT is performed through a series of four separate, interacting research teams. The tasks listed below in many cases depend on or feed into research performed by other teams.

Biomass Production Team

1) Develop a Plant Production Decision Support System Assisted by Machine Vision Monitoring of Plant Stress.
- Identify for feature extraction spectral quality change and quantify morphological change due to temperature-induced stress of tomato plants.
- Develop an integrated sensing system for plant development monitoring.
- Establish automated control algorithms correcting the effects of the variances for management of a biomass production sub-system (PBS) within a Bioregenerative life support system (BLSS).

2) Investigate Environmental Control of Tomato Production: Temperature Effects on Growth, Yield and Fruit Quality.
- Investigate the effects of perturbations in air temperature on tomato growth, physiology, and yield.
- Correlate fruit quality parameters with temperature changes and whole plant growth and developmental state.
- Establish a relationship between altered sink strength and AGP gene(s) expression.

3) Modeling and Optimization of Biomass Production Systems Including a Hypermedia Application.
- Collect data and existing models as inputs to the Subsystem modeling process.
- Develop an application that will serve as both a decision-making tool and a computer-based testing vehicle that will facilitate training.

Food Processing and Nutrition Team

1) Plan and coordinate a conference to define research needs for nutritional issues related to long-term space flight. The information generated by this conference will be used to further define the nutritional needs of space voyagers in stressed, low-gravity conditions and to develop palatable menus for space voyagers and recommend plants for a BLSS to ensure a complete supply of all essential nutrients.

2) Use the crops that have been selected by ALS investigators to produce a palatable and nutritious menu for long-term space flight. Studies will begin with soy beans and wheat to produce a variety of ingredients.

3) Develop mathematical simulation necessary to develop a versatile and miniaturized extruder suitable for a life support environment.

Waste Processing and Resource Recovery

1) Volatile Organic Air Contaminants: Identification, Monitoring and Control.
- Identify and quantify amounts of volatile air contaminants that will enter a Bioregenerative Life Support System (BLSS) atmosphere from: humans, plants, food processing and waste treatment and from a database of these contaminants.
- Develop regenerative treatment methods for removing those volatile contaminants from the BLSS atmosphere.
- Develop a continuous monitoring system that can be used to control operation of the regenerative treatment system and thus ensure the maintenance of satisfactory air quality on the BLSS.

2) Recovery of Nutrients from Non-Edible Plant Material.
- Characterize the non-edible portion of tomato plants.
- Compare the results of heat treatment and nutrient addition on the rate and efficiency of the degradation process.
- Construct and operate a bench-scale anaerobic/aerobic treatment system.

3) Non Linear Empirical Modeling and Optimization of Waste Recovery Systems.
- Machine readable input-output data for a variety of waste treatment processes.
- Provide input for "Global Optimization" project to Systems Studies and Modeling team.

Systems Studies and Modeling Team

1) Automation-Culture-Environment Oriented Analysis for Bioregenerative Life Support Systems.
- Establish an NJ-NSCORT computer network to facilitate operations analysis capability to support planning, analysis, requirements definition, design, and possibly control of a BLSS.
- Perform a systems abstraction and formulate description of operation schemes within a BLSS
- Develop system requirements and database and software framework for ACE_SYS.

2) Global Optimization of a Complex Integrated Systems for Bioregenerative Life Support.
- Review existing models related to systems integration, analysis and information flow within a BLSS.
- Define key variables that interact with other modules in a BLSS.
- Collect data and models from principal investigators and research associates in each project and generate polynomial submodels based upon that information.

The New Jersey NASA Specialized Center of Research and Training for Bioregenerative Life Support Systems began operating on May 1, 1996. The following progress was recorded by each team before September 30, 1996.

Biomass Production Team

1) Machine vision system developed to automatically monitor the development of the tomato seedling.
2) Growth chamber environmental temperature effects on tomato growth have been manually monitored for correlation with machine vision automation.
3) The development of a transgenic plant with gene for an enzyme that promotes starch synthesis and ultimately total soluble solids within the tomato fruit is in progress.
4) Studies begun for the use of plant growth regulators for modulating shoot development and concentrating flowering have identified a fatty acid methyl ester as a potential chemical pruning agent that reduces side shoot biomass and the need for manual pruning.

5) The architecture of the Single Truss Tomato Production System (STTPS) decision support system software has been developed. This framework utilizes process and management information on the STTPS in operation at the Burlington County- New Jersey EcoComplex Demonstration Greenhouse.

Food Processing and Nutrition Team

1) Designed and built extruder die with active heating and long channel toproduce different textures based on wheat flour/soy flour blends.
2) Selected products, including breakfast cereal, crispy bread, pasta and chips, to be produced with single-screw extruder using different feeders, dies and screws. To achieve variety, examined vapor release extrusion for expanded products and non vapor-release for dense, non-porous products.
3) Conducted extrusion experiments using what flour/soy flour blends to create desired textures and functionality. Parameters used were temperature, RPM and flour blends.
4) Collected literature review on image analysis of texture of porous non-food materials, e.g. water-holding capacity of foods.
5) Prepared preliminary outline and agenda for symposium that will identify gaps in nutrition research for long-term space travel and set priorities to fill those gaps.

Waste Processing and Recycling Team

1) Designed and constructed apparatus to sample headspace around tomato plant.
2) Analyzed samples using thermal desorption-GC-MS; major volatile emissions were terpenes and sesquiterpenes.
3) Biofilter experiments showed ammonia in air can be biologically converted to nitrate. Perlite chosen as packing material for biofilter.
4) Characterization of tomato plants completed: water content, cellulose content, minerals.
5) Preliminary respirometer test conducted to assess biodegradability of tomato plant.
6) Heat treatment used to break down complex structure: aerobic biodegradation study performed in shaker flasks.

Systems Studies and Modeling Team

1) An integrated cyber (client-server applications) environment for NJ-NSCORT research teams has been specified and implemented.
NJ-NSCORT Home Page <http://www.rci.rutgers.edu/~biorengg/njnscort>
ACE_YSYS-B <http://nj-nscort.rutgers.edu/acesys>
2) Discussion sessions with NJ-NSCORT research projects have been conducted to determine their information needs and inputs .
3) Client-server application programs are being developed to extend communication channel among NJ-NSCORT research teams and establish systems analysis capabilities.
4) Multiple polynomial regression models are being developed to qualify waste treatment processes.

NSCORT research on agricultural efficiency, food processing and waste management will help solve problems we face today in our farms, factories and backyards. NSCORT research is particularly valuable in urbanizing areas, which must solve problems of declining farmland, waste management and agricultural profitability.

Limited resources and agricultural productivity

NJ-NSCORT is developing methods to maximize the production of edible crops in an enclosed area while at the same time conserving and recycling as much of the water and nutrients as possible. The machine vision system developed by NJ-NSCORTís biomass production team offers a way to continuously monitor crop performance. We also are generating the data necessary to develop a crop growth model that predicts the effects of temperature changes on harvest date.

Our food processing team is developing a model to better predict how small-scale food extruders will work. Extrusion is a versatile food processing system that simultaneously mixes, cooks and shapes food. The end product of this work could be a home-scale food extruder that would allow families to create a wide variety foods ñ from customized breakfast cereals to breads and chips.

Environmental Management

Our waste management team designed a new apparatus consisting to sample the headspace around plants for trapping and measuring volatile compounds. A second group within our waste management team has designed a biofilter to remove ammonia from air and design ofbiofilter for ethylene removal has begun. A third group within the waste management team has conducted preliminary experiments to maximize recovery of nutrients from the non-edible portion of plants, which will help increase the attractiveness of recycling of this portion of the organic waste stream.

NSCORT: Gravitational Biology

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Funding:

Project Identification: 199-93-17-13
Initial Funding Date: 1/96
FY 1996 Funding: $867,248

Solicitation: 94-OLMSA-04
Expiration: 12/00
Students Funded Under Research: 22

Task Description:

The NASA Specialized Center of Research and Training (NSCORT) in Gravitational Biology at Rice University's Institute of Biosciences and Bioengineering was officially initiated March 1, 1996. We are pleased to submit this report on the progress made during the first year of our NSCORT and our plans for the coming year.

MISSION STATEMENT

The mission of our NSCORT is to investigate the effects of gravity and other environmental factors on biological function at the cellular and molecular level. The research efforts, training opportunities, and scientific exchange will promote the expansion of a scientific peer group well-educated in space-related biological issues. This will stimulate the interest of the larger scientific community and insure the continuing development of rigorous flight investigations in Gravitational Biology.

RESEARCH OVERVIEW

The Rice NSCORT research program is focused on examining the effects of microgravity and associated stresses on development and cell culture in prokaryotes and eukaryotes. While most of the research involves mammalian cells, exciting research is also underway on plant cells, yeasts, microbial cells and *Drosophila melanogastor*.

Many of the projects include utilization of the rotating bioreactor systems developed at Johnson Space Center as a tool for simulation of some aspects of the microgravity environment.

Several of the project principal investigators have established research programs centered on understanding the molecular basis of the response of various cell types to mechanical stimuli. These faculty include Professor Braam (employing a plant model), Professor Gustin (using yeast) and Professor McIntire (mammalian cells). The NSCORT research program is centered on understanding at the molecular and cellular level, the effect of microgravity and associated stresses on development and cell function. Drosophila are chosen as the model system for two of the developmental biology projects because of the wealth of molecular level knowledge and reagents that are available to allow testing of very specific mechanistic hypotheses (Projects I and II). In addition, this model system is relatively easy to translate to actual flight experiments once ground-based research identifies the proper questions to ask and establishes the requisite data base for comparison.

Projects III, IV and V examine basic cellular mechanisms involved in sensing the mechanical force environment, in transduction of these signals, and in gene regulation, using arabidopsis, yeast and mammalian cell models. These projects interact closely with each other because of their common scientific interest in signal transduction and the understanding produced forms a mechanistic basis for developing hypotheses concerning the effect of microgravity on cell function. The data produced will also have wide application in many other areas where mechanical forces are intimately coupled with gene regulation and cell metabolism.

The remaining four projects currently underway examine specific hypotheses focused on understanding the molecular and cellular basis of several documented cellular alterations seen in space flight. These include a dramatic reduction in lymphocyte DNA synthesis in response to mitogens (Project VI) and in migration (Project IX), increased bone resorption and demineralization (Project VII), and skeletal muscle atrophy (Project VIII).

The model systems have been carefully chosen so that specific mechanistic questions at the cellular and molecular level can be addressed and so that once the proper ground-based data have been assembled, the development of flight based proposals will not be difficult.

These projects span a sufficient range of areas to be attractive to a wide range of potential trainees - undergraduate, graduate students and postdoctoral fellows. Too narrow a focus would limit the attraction of the NSCORT and we would not be able to interest the highest quantity young scientists and engineers we are currently attracting.

The goal of the NSCORT at Rice University is to develop a high quality training and research program that will establish a NASA-university base for space-related research. The research emphasis will be in the area of Gravitational Biology, in particular the investigation of the effects of gravity and other environmental factors on biological function at the cellular and molecular level. The research efforts, training opportunities, and scientific exchange will promote the expansion of a scientific peer group well-educated in space-related biological issues. This will stimulate the interest of the larger scientific community and ensure the continuing development of rigorous flight investigations in Gravitation Biology.

There are twelve major research projects which span a wide range of interests. The model systems have been carefully chosen so that specific mechanistic questions at the cellular and molecular level can be addressed and so that once the proper ground-based data have been assembled, the development of flight based proposals will not be difficult.

RESPONSES TO MECHANICAL STIMULI: Several of the project principal investigators have established research programs centered on understanding the molecular basis of the response of various cell types to mechanical stimuli. These projects examine basic cellular mechanisms involved in sensing the mechanical force environment, in transduction of these signals, and in gene regulation, using arabidopsis, yeast and mammalian cell models. They interact closely with each other because of their common scientific interest in signal

transduction, and the understanding gained forms a mechanistic basis for developing hypotheses concerning the effect of microgravity on cell function. The data produced will also have wide application in many other areas where mechanical forces are intimately coupled with gene regulation and cell metabolism.

DEVELOPMENTAL BIOLOGY: Another focus area in the NSCORT is centered on understanding the effect of microgravity and associated stresses on development at the molecular and cellular level. Drosophila are chosen as the model system for these developmental biology projects because of the wealth of molecular level knowledge and reagents that are available to allow testing of very specific mechanistic hypotheses. In addition, this model system is relatively easy to translate to actual flight experiments once ground-based research identifies the proper questions to ask and establishes the requisite data base for comparison.

OTHER SYSTEMS: The remaining seven projects examine specific hypotheses focused on understanding the molecular and cellular basis of several documented cellular alterations seen in space flight. These include a dramatic reduction in lymphocyte DNA synthesis in response to mitogens and in migration, increased bone resorption and demineralization, and skeletal muscle atrophy. Finally, two exciting basic research projects on understanding microgravity effects on cytoskeletal assembly in mammalian cells (both in suspension and for attachment-dependent cells) and effects of microgravity on microbe-host interaction will be undertaken.

NSCORT: BIOREGENERATIVE LIFE SUPPORT - Biomass Productivity and Sustainability of Bioregenerative Life Support Systems

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Funding:

Project Identification: 199-93-17-03
Initial Funding Date: 1/91
FY 1996 Funding: $397,519

Solicitation: NRA, 1990, NSCORT
Expiration: 12/95
Students Funded Under Research: 8

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 were 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, for 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.

In the higher plant biomass productivity laboratory of Dr. Mitchell, progress was made on three projects.

Graduate student Rachelle Goldman found that day-neutral, semi-dwarf rice (*Oryza sativa L.*) was responsive to photoperiod shifts in terms of grain yield parameters that are important for crop production in bioregenerative life-support systems. Although edible yield rate (g DW grain•m-2•day-1) increased with exposure to continuous light before transfer to 8-h or 12-h photoperiods (because number of tillers and panicles per plant increased), the shoot harvest index declined for both photoperiod shift treatments. This occurred because, as competing vegetative sinks on each plant in a stand increased, the proportion of infertile panicles on each plant also increased. Due to the need to recycle non-edible biomass to renewable resources in a CELSS, partitioning between grain and vegetative growth is as important as absolute yield. Ultimately, Goldman found that a straight 12-h photoperiod regime throughout production was superior to long → short or short → long photoperiod shifts, or a straight 8-h photoperiod regime. Goldman also surveyed a wide range of stand densities for rice at different photoperiods and concluded that 280 plants•m-2 under 12-h photoperiods was optimum for a range of productivity parameters including grain yield, cropping time, growth area, and non-edible biomass residue. Growth chamber studies indicated that high light early in the cropping cycle determined yield much more strongly than during the post-anthesis, grainfill period, indicating that electrical energy can be saved during the latter period. She also found that rice thrives on NH^{4+} nitrogen in hydroponic culture with regular pH adjustment, indicating that direct use of nitrogenous wastes rather than relying upon nitrification is feasible for CELSS.

In the Post-Doctoral project of Dr. Changhoo Chun, a real-time dynamic feedback control system is being developed in which crop canopies tell a trained computer what their current photosynthetic preference is for light, CO_2, and temperature. Photosynthetic photon flux (PPF) is manipulated by electronic dimming ballasts connected to a computer through an A/D or D/A input-output mother board; CO_2 is controlled by a mass-flow control system through the same device. Decision making is being developed that combines empirical mathematical models with rule sets developed from recent growth and gas-exchange data obtained from leaf lettuce canopies grown in the minitron II Plant Growth/Canopy gas-exchange system. Lettuce (*Lactua sativa L.*) is a rapid-cycling vegetative crop whose lag and exponential growth phases are good models for reproductive

crops during their vegetative growth stage. The goal of this research is not to maximize productivity but to optimize output/input ratios. Electrical power (kW/m^2) and energy consumption ($kW\text{-}h/m^2$) are considered as crop production costs and are factored into crop production protocols. For example, during lag and plateau phases of lettuce growth, there is much less positive photosynthetic rate (Pn) response to increments of PPF and/or CO_2, so canopies are challenged incrementally or decrementally at regular time intervals with these environmental inputs, and the optimum Pn/kW ratio is selected. In this way, energy efficient "optimizing environments" are selected naturally and seem to be important especially during the first few days of exponential growth. Initial operator-assisted dynamic optimization protocols using variable CO_2 and variable PPF indicated 32% more efficient electrical energy conversion into edible biomass than using static high CO_2 and high light. Full computer automation of this approach has potential for even greater resource savings in controlled environment crop production.

Graduate student Jonathan Frantz is investigating the feasibility of using low-intensity intracanopy lighting as an energy-efficient alternative to high-intensity overhead lighting in the production of closed crop canopies. Frantz and Chun set up vertical or horizontal orientations of low wattage, mylar-sleeved fluorescent lamps within separate compartments in two separate growth rooms, one overhead lighted and the other not. Without intracanopy supplementation, the dark interior of overhead-lighted cowpea (*Vigna unguiculata Walp.*) canopies was devoid of leaves, pods, or flowers. When intracanopy lighting provided the sole source of photosynthetically active radiation, the canopy was able to flower, set seed, and produce mature pods. Although edible yield rates (of young leaves plus seeds) were a third of those using only overhead lighting, yield-efficiency rates (g edible$\cdot m^{-2}\cdot day^{-1}\cdot$g inedible^{-1}) were 3 times more for intracanopy lighting only. Leaf/stem biomass ratios also were 3 times more for intracanopy than for overhead lighting, while harvest index (reproductive + vegetative) was double. Although the kW-h/g total biomass averaged the same for both types of lighting, the input energy for edible biomass production was only half as much for intracanopy lighting. Factoring the crop production penalties of electrical energy, non-edible biomass, and cropping time together, the results to date indicate an overall efficiency improvement of about 12 times using intracanopy rather than overhead lighting. Present studies testing the effects of reflective films or light-scattering plastics figure to make intracanopy lighting even more effective.

Dr. Mark Schneegurt completed a post-doctoral appointment in the Algal Biomass Productivity laboratory of Dr. Louis Sherman. The work related generally to the use of Cyanobacteria in a CELSS, including atmospheric balance of O_2, CO_2, and N_2. This work was then embedded in a comprehensive model of CELSS termed CELSS 3-D using the program STELLA. This model showed how plants and cyanobacteria could provide the atmosphere and food for 8 crew members on a 3-year voyage to Mars. This was a complex endeavor involving data from all members of the Center. These results were written for publication and submitted to *Life Support and Biosphere Science*. This was a culmination of the work done by the Sherman lab as part of the NSCORT project and represents one of the more comprehensive biological models developed for such life-support systems.

Close-out funds obtained from NASA were in used in the Food Science laboratory of Dr. S. Nielsen to complete the work of graduate student Deb Smart, who worked on effects of environment and crop maturity on the chemical composition of select CELSS candidate species. Select CELSS candidate crops gown in the field or in controlled environments were analyzed for total nitrogen (N), protein N, nitrate, amino acids (total, free, and peptides), fat, ash, individual minerals, total dietary fiber, cellulose, lignin, hemicellulose, and oligosaccharides (raffinose, stachyose, and verbascose). Differences were detected between free amino acid levels of field-grown and controlled environment-grown wheat and potatoes. Carbon dioxide level in controlled environments greatly affected the chemical composition of cowpea and soybeans. Oligosaccharide content increased in cowpeas seeds during maturation, while protein concentration on a dry weight basis remained constant. The chemical composition of soybean and wheat plants also was affected by maturation. As chemical composition changes due to growing environment and maturity are identified, researchers will be better able to design safe and nutritious diets for astronauts while minimizing resources needed.

In the Food Processing group of Dr. John Floros, Post-doctoral Research Associate Dr. Linus Fonkwe investigated effects of soaking conditions (temperature, pH and time), and the use of sodium phosphate as

soaking medium, on the quality of soymilk (pH, color, relative density, and oligosaccharide content) during the processing of soybeans. Graduate student Jerry Farkas determined the effectiveness of oxygen scavengers and headspace flushing in retarding lipid oxidation in oil, while graduate student Sandra Lay examined the ability of modified atmospheres combined with preservatives to extend the shelf life of fresh fruit and vegetable tissue.

In Dr. Belury's Foods and Nutrition laboratory, Post-doctoral Research Associate Kwangok Nickel investigated the nutritional adequacy of CELSS vegetarian diets focussing on mineral bioavailability, especially of calcium, as well as bone status using a rat model. She found that bone health (i.e., strength, flexibility, density) of subjects fed unsupplemented vegan diets was equivalent to that of animals fed control diets, indicating no major calcium bioavailability problems with CELSS diets composed from edible biomass grown in controlled environments.

In Dr. Hodges' Biotechnology laboratory, two projects were continued that had been undertaken with the NASA funds. The first project was on modification and expression of the glutelin gene in rice. The second project concerned an attempt to alter the lignin content of rice. In both studies, efforts were being made to make binary vectors that contained the modified glutelin gene or the antisense of the gene that makes an intermediate in the synthesis of lignin for insertion into *Agrobacterium*, which would then be used to transform rice. They are still working, with no funds, on making the vectors containing the modified glutelin gene. This has caused problems because this gene is difficult to amplify in bacteria. For some reason, this modified gene impairs bacterial growth making the manipulations difficult. The antisense gene constructs that interfere with lignin biosynthesis were developed to an intermediate stage before the funds ran out, and they are stored in a -80°C freezer until new funding is obtained to continue this project.

Dr. Auslander's Systems Analysis group submitted a paper to the journal Operations Research entitled "Biologically based human diet selection under complex scenarios using a fuzzy logic knowledge base." This paper represents an extension of their earlier work on the diet selection problem (Eisenberg et al. 1995). The paper describes the use of a fuzzy logic knowledge base to capture dietary requirements, which can then be used to generate diet strategies. The fuzzy logic knowledge base accommodates uncertainty and variability inherent in the expression of these biological constraints and permits the comparison of candidate diets. The second area of effort has concentrated on the identification of a suitable theory for the management of advanced life support (ALS) subsystems within a control hierarchy. Internal requirements will impose constraints on the individual operation of ALS subsystems. The interconnection of these in a working life-support system will require that subsystem inputs and outputs meet constraints imposed by global system requirements. The consideration of both types of constraints at the subsystem level will be necessary to realize hierarchical control of ALS systems.

As Purdue NSCORT Education and Outreach Coordinator, Bonnie McClain's activities included numerous presentations at schools within the state of Indiana and for elementary and middle school classes visiting the Purdue campus. The Purdue Visitor's Center requested an average of two talks per month for visiting groups. Response to inquiries by e-mail and regular mail was constant. Facilitating a grant proposal "Cells in CELSS: A Life Systems Study" proved successful and the Space Grant Consortium Mini-grant was awarded to develop a series of nine lessons based on the NSCORT research. Development of two slide presentations "Come Dine with Me in Space" and "Come Dine with Me on Mars" were completed.

In January, 1996, Bonnie began transitioning responsibilites that would become a part of her new position as a grantee to NASA Life Sciences Education and Outreach. Communication with all 8 NSCORTs was established and assistance was given to all NSCORTs to develop or enhance their NSCORT Education and Outreach programs. Bonnie was named the NASA Life Sciences Education Lead Person for the following: NASA Specialized Centers of Research and Training, The Collaborative Ukrainian Experiment Education Project, the Purdue Space Grant Consortium Mini-grant, and the Science-By-Mail Project. She was named the Life Sciences Education Liaison to the NASA Education Division, the Neurolab Education Project, and the Aerospace States Association. She serves as the coordinator for the NASA Life Sciences exhibit area and presentations for the National Science Teachers Conference and for the Colorado Space Education Initiative Conference. Bonnie continues to be a featured speaker at space education conferences and often speaks at schools for convocations and

individual classroom visits. Her talk "Come Dine with Me in Space" has been presented to a variety of audiences including: Mensa Regional Gathering, Colorado Space Education Conference, International Space Station Educators Conference, Great Lakes Region Aviation and Space Education Conference, and elementary and middle school students.

Work conducted by the Purdue NSCORT in Bioregenerative Life Support from 1990 through 1995, and during the 1996 closeout period addressed the issue of "sustainability" of a bioregenerative life-support system. The general approach was to identify processes and protocols that would permit development of a recycling life-support system that could operate stably within reasonable constraints of power, labor, mass, volume, and leak rate for long periods of time. Efficiency of candidate processes was an emphasis of research in the Purdue NSCORT.

There are many potential spinoffs from each of the major research projects of the Purdue NSCORT, but the relatively brief window of funding has precluded development of any of them to a useful end point. The nature of the potential spinoffs is detailed in a publication co-authored by the NSCORT faculty (Mitchell et al. 1996. Earth benefits of interdisciplinary CELSS-related research by the NSCORT in bioregenerative life support. *Adv. Space Res.* 18(4/5):23-31). Briefly, Earth benefits from this NSCORT would have included the following: development of dynamic optimization mechanisms for the energy-efficient control of light, CO_2, and temperature in the hydroponic, controlled environment production of CELSS candidate crop species; guidelines for using cyanobacteria to maintain O_2, CO_2, and N_2 balance within a complete CELSS system; development of transgenic cereal and legume crops no longer deficient in essential amino acids and needed for a balanced vegetarian diet; use of anti-sense RNA technologies to create low non-edible crop residues during crop production; learning how to control nutrient/anti-nutrient/toxin contents of edible crop biomass by manipulation of crop production environments and mineral nutrition; development of food-process procedures to remove/destroy anti-nutrients from crop biomass; development of food-process technologies and equipment for use in non-extensive closed systems; development of rapid microorganism-based technologies for bioconversion of organic wastes to renewable resources or intermediate, novel food products; and development of control system strategies for complex systems with potential for chaotic behavior of subsystem components. Although publications coming out from Purdue NSCORT work in each of these potentially valuable spinoff areas will benchmark progress made toward realizing these goals, the loss of interdisciplinary synergism will greatly increase the timeline and expense required for their eventual realization.

FY96 Publications, Presentations, and Other Accomplishments:

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NSCORT: Vestibular Research/(NIH)

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Funding:

Project Identification: 199-93-17-09
Initial Funding Date: 8/95
FY 1996 Funding: $500,000
Joint Agency Participation: NIH

Solicitation:
Expiration: 8/98
Students Funded Under Research: 6

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 One and Two will bring on line new devices designed specifically to study otolith systems. Modelers involved in Projects One, Two, Four and Five 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 Two 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 One and Four share the use of an elegant new biomechanical model that allows one to construct accurate models of musculoskeletal systems whose kinetic properties can then be simulated under a wide variety of conditions. Projects One and Five employ non-linear systems models to simulate how central nervous system control of head or body

position interacts with body biomechanics. Project Two 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 ONE: ANGULAR AND LINEAR VESTIBULOCOLLIC REFLEXES IN HUMANS. We have completed collection of open loop VCR and CCR data in intact subjects and begun an investigation of the closed loop VCR and CCR in subjects with complete bilateral labyrinthine loss. We have also begun studies to examine the dynamic properties and behavioral modulation of the linear VCR using our linear sled. Subjects exhibited both an expected first-harmonic response, and a prominent second harmonic response, that appears to be predictive in nature. We are developing a sliding frequency, anterior-posterior linear velocity "Chirp" stimulus, to probe this behavior further.

In this year's dynamical modeling efforts, we have extended our single joint pitch model from last year into a considerably more complex 2 joint pitch model. We have also significantly advanced in our efforts to prepare a biomechanically accurate model of the human head-neck system using neck muscle architectural parameters collected experimentally through collaborations with Queen's University in Kingston, Ontario. Model estimates of maximal isometric moments exerted by each neck muscle led to the interesting finding that the classically defined neck muscles are incapable of accounting for human motor performance in flexing the head so that actions of muscles such as infrahyoid muscle (not usually considered to be a neck muscle) need to be included. Supporting this idea, preliminary experimental data have revealed that the infrahyoid muscle is strongly activated during flexion moments. Examination of physiological operating ranges of neck muscles showed that they vary considerably among movement directions and can approach the limits where force production is reduced. Potentially, many neck muscles may lose much of their force generation capacity when the head position approaches the ends of its range of motion. Thus, characterizing the interaction of neck muscle architecture and musculoskeletal geometry is likely to be important to understand muscle moment-generating and stabilizing capability at different head postures.

PROJECT TWO: LINEAR VESTIBULOCOLLIC REFLEX IN PRIMATES. We are progressing in our studies of electromyographic responses during reflex excitation of neck muscles during externally applied linear and rotational motions. The focus of work this year has been the spatial reorganization of squirrel monkey vestibulocollic reflexes when the body is re-oriented with respect to gravity. The salient finding is that the direction of neck muscle excitation reverses when the head is inverted with respect to gravity. This means that the direction of the vestibular neck reflexes is reversed so that they become anti-compensatory in the inverted head posture. Two possible, related explanations of this result are being explored. First, it could be that the compensatory vestibulo-collic reflex which operates in the normal upright posture competes with a positive-feedback anti-compensatory righting reflex when the posture is far from the normal position. Second, it could be that the stability of the head provided by gravity when the head is inverted, is accompanied by shut-down of the then unnecessary stabilizing reflex. Instead, when the head is inverted it may be too stable (too hard to move into a different posture), and a reversed reflex is needed to set head stability at a level similar to that in the upright posture. 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 THREE: HEAD STABILIZATION IN THE MONKEY. Significant progress has been made during the last year. One paper has been published, another was presented at the Barany Society in August, and three

presentations were given at the Society for Neuroscience in November. We are currently analyzing our data, developing and conducting experiments designed to elucidate the underlying neural mechanisms responsible for the dramatic modification of firing rate of secondary vestibular during voluntary head movements observed in our studies, and are preparing several reports for journal submission. The main findings that are to be published are similar to those mentioned in last year's report but greatly elaborated; they relate to 1) the gain changes in the vestibulo-collic reflex linked to active gaze pursuit, and 2) the disparate signals on vestibular neurons, including identified vestibulospinal neurons, during externally applied or passive and self-generated or active head movements.

PROJECT FOUR: ORGANIZATION OF POSTURAL CONTROL: VESTIBULAR PROCESSING. This project aims to identify the contribution of otolith and canal signals as well as biomechanical constraints to postural control strategies. This year, we have applied our understanding of postural coordination in healthy subjects to patients with severe, bilateral vestibular loss in order to relate otolith and canal function to specific aspects of postural coordination. In collaboration with Dr. Peterka, we found that patients with severe, bilateral loss of horizontal canal vestibular function could show relative preservation of otolith and vertical canal function as tested by measurement of vestibuloocular reflexes with off-vertical axis and pitch plane rotations. Postural coordination was shown to be abnormal in these patients with loss of vestibular function in two tasks: 1) selection of a hip strategy in response to large, fast surface translations when vestibular loss occurs early, but not late, in life; and 2) control of the trunk orientation and stability during sinusoidal oscillations of the support surface with eyes closed. Contrary to our earlier hypotheses, we found that vestibular function is not necessary to trigger a hip strategy but it may be critical during infancy to establish normal hip patterns. We also report similarities and differences in biomechanical and neural constraints on postural control in the sagittal and frontal directions. Sagittal and frontal postural responses showed similar kinematic and surface force strategies but different muscle synergy patterns with early trunk control for regulating lateral posture and early ankle control for regulating sagittal posture. The critical role of somatosensory information in triggering postural responses was revealed in a very rare patient with total body somatosensory loss since automatic responses both to external and to self-generated perturbations were absent. Nevertheless, this patient was able to stand independently and resist perturbations by longer-latency substitution of auditory and visual cues when perturbation direction was predictable

We are also expanding our model of postural control to include a process for adaptation of postural control for altered sensory environments. The model suggests that use of an internal, neural model of expected sensory information, which may relate to cerebellar function, can be used to predict postural behavior under altered sensory conditions. Multivariate descriptors of human postural sway are useful to fully characterize postural stability under altered sensory conditions and are being tested in patients with either vestibular loss or somatosensory loss from diabetic peripheral neuropathy.

PROJECT FIVE: VESTIBULOSPINAL CONTROL OF POSTURE AND LOCOMOTION. The aim of this project is to investigate the role of the vestibular system in maintaining balance during stance in the cat. The focus is on stabilization of the head and trunk during quiet stance and during responses for postural equilibrium. An x-ray and modeling study of the vertebral column, completed this year, has revealed i) new insights about trunk and forelimb configuration and, ii) a dramatic revision of the identity of antigravity muscles of the head and trunk. A functional understanding of these muscles is important because they are the muscles most likely to receive descending inputs from otolith-spinal neurons. We predict it is these muscles that will be most strongly affected by vestibular dysfunction. These studies provide the critical underpinnings for our aim to quantify the effect of vestibular lesions on postural orientation and equilibrium. Progress was also made in several technical areas, including: i) incorporation of a 6D magnetic tracker (Polhemus) for accurately recording head position, ii) calibration of the head recording system to match the coordinate system of body kinematics (using Optotrak), iii) software development and modifications for collection and analysis of head position data, iv) development of a setup for overground locomotion in the cat, including kinematics, EMG, and ground-reaction forces from five force plates, and v) incorporation into the lab of the methodology for microinjections into the brainstem of awake, behaving cats.

Projects One and Four, which examine vestibular reflexes in humans, are yielding information that will help us understand and treat disorders of balance and posture. Proper control of the head-neck motor system during rotations and translations of the body is essential for controlling the orientation of the head's special sensory receptors in space and regulating the attitude of the head on the trunk as part of overall postural control. Such control can be seriously degraded in patients with vestibular or neurological abnormalities. Work related to Project One is helping us to understand the problems experienced by such patients and is suggesting ways in which they could be ameliorated.

It is not yet possible for clinicians to accurately diagnose disorders of the vestibular otoliths in humans, since their role in vestibulo-spinal behavior is unclear. Studies carried out under Project Four suggest that vestibular information, particularly otolith information, may be critical for control of specific types of postural tasks under specific sensory and biomechanical constraints on balance, 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 four of Project one is making good progress toward obtaining the first biomechanically accurate model of the human head-neck system. Given the great interest in this system from the standpoint of human factors and whiplash studies, it is surprising that no such model exists in the scientific literature to date. 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 One and Four are also providing information on basic biological processes involved in converting input from receptors of the vestibular labyrinth into motor commands required to maintain postural stability. A wealth of additional basic biological information is being generated by Projects Two, Three and Five, which examine vestibulo-spinal systems at levels of detail that are not possible in humans. Projects Two and Five are revealing the detailed patterns of reflex muscle activation that underlie postural stabilization. Project three is revealing exciting new aspects of central neural processing that allows neurons of the vestibulo-spinal system to differentiate between vestibular afferent signals generated by voluntary head movements and passive displacement of the body — an attribute of CNS processing that is likely critical for accurate postural regulation.

With their common emphasis on processing of vestibular afferent signals, especially those arising from gravity-sensitive otolith organs, these five projects also have obvious relevance to space flight. Under microgravity conditions on orbit, the reflex stabilizing systems we are studying must be modified to maintain proper motor control. Such modifications must then be reversed upon return to Earth. Residual adaptive changes likely account for many of the postural problems experienced by astronauts immediately after landing. Our results should help to understand and remediate these problems.

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NSCORT: The Center for Gravitational Studies in Cellular and Developmental Biology

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Funding:

Project Identification: 199-93-17-01
Initial Funding Date: 1/91
FY 1996 Funding: $175,000

Solicitation:
Expiration: 12/95
Students Funded Under Research: 65

Task Description:

The proposed focus of this Center is in the area of gravitational biology with the research emphasis in cellular and developmental biology. The research component of the Center has been developed in light of the existing information available on the role of gravity in cellular and developmental biology. It is already clear that reduced gravity has a significant impact on some cell types and cellular activities, and on some developmental systems and processes. However, a comprehensive and focused analysis has not yet been conducted. The individual projects proposed for this Center are diverse in that they include studies on higher plant, protozoan, yeast, insect, avian, and mammalian systems. The particular feature of the research effort that relates to such diversity is our unifying hypothesis that the cellular cytoskeleton and the extracellular matrix (ECM) represent gravity sensitive macromolecular assemblages. When viewed from the standpoint of this hypothesis, the diversity of the research systems being used becomes particularly advantageous as a comprehensive approach to analysis of the impact of gravity on cellular and developmental biology. It is clear that the plasma membrane is central to the regulation of cellular activities. Thus, incoming signals, whether from distant sites (like hormone or growth factor ligands) or from the local environment (like ECM molecules), bind to integral membrane receptors. Signal transduction events routinely involve the cellular cytoskeleton, either directly or indirectly. The cytoskeleton is composed of several dynamic macromolecular assemblages crucial to control of cell division, cell motility, cell shape, and endo- and exocytosis. Thus, the cytoskeleton is crucial to the cellular processing of incoming information, which leads to the generation of a cellular response. When the response involves morphogenesis, differentiation, or cell division, the cytoskeleton is again centrally involved. If secretory activity or exocytosis is part of the response, the cytoskeleton is essential. Therefore, the cytoskeleton also controls outgoing information. Furthermore, secretions into the local environment modify the ECM both by assembly and degradation. In both cases, the altered ECM represents a new set of signals to interact with receptors on the cell surface, producing yet another cellular response. The ECM, therefore, is a dynamic macromolecular assemblage whose state and degree of organization is also crucial to cell motility, cell division, morphogenesis, and differentiation. The plasma membrane, which houses receptor and signal transduction proteins, is the intermediary between the proposed gravity sensitive intracellular cytoskeletal compartment and extracellular ECM compartment. Each of the projects proposed for this Center addresses some aspects of this system and will serve to elucidate our understanding of gravity in cellular and developmental biology. The unique strength of the research component is in the combination of our unifying hypothesis and our selection of faculty scientists and projects with diversity, breadth, and depth that will ensure a systematic and serious test of that hypothesis.

The research conducted in this Center addresses fundamental questions regarding the role of gravity in cellular and developmental biology. The progress made on specific research projects has substantial potential value to humankind on Earth, as well as to a manned presence in space. While the research is basic in nature, there are enormous potential benefits. Some examples of areas of impact include: Immune cell biology — These studies are of value in understanding the immune system in normal and compromised situations, as experienced both on Earth and in space, and have potential in understanding of immune cell interactions, cytokine production and regulation, and potential therapies for correction of disease states and altered physiological states. Plant developmental biology — These studies have potential impact in the general areas of agriculture and food production and quality, and physiological understanding of gene regulation in harsh environments, such as closed systems, non-optimal gas, light, temperature, and gravity situations, as can be found on Earth and during space flight. Eye development — This research impacts on understanding the structure, function, development, and gene regulation in the vertebrate eye, and has major potential benefit in understanding of, and possible therapies for, various eye diseases, including cataracts, keratoconus, and optic dysfunction. Embryonic organ development — These studies have potential impact on understanding of abnormal development and birth defects, and specific disease and dysfunctional organ situations, including lung (respiratory distress syndrome), pancreas and salivary glands (digestive enzymes, exocrine function, endocrine function, diabetes), heart formation (congenital defects, myocardial disease, circulation), and skeletal tissue formation (osteoporosis).

Information regarding specific progress made during FY96 was not provided by the principal investigator.

Appendix